

**Modulating the glycaemic response of ready to eat
extruded snack products utilising dietary fibre and
fibre rich waste stream materials.**

**Presented in partial fulfilment of the requirement for the degree
of**

Ph. D.

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Abstract

The aim of this Ph. D. was to utilise commercial dietary fibre (DF) sources as well as DF from food waste streams to create snacks capable of reducing glycaemic response (GR). Obesity is a rising global epidemic due to changes in lifestyle, eating and exercise habits. Consumer demand for convenience has led to greater consumption of highly processed and refined foods so that even though cereal consumption is still high, many of the associated phytochemicals are removed, creating snacks high in energy and low in DF. High energy, low DF, diets have been linked to diabetes, certain types of cancer and heart disease. Health conscious consumers are demanding 'healthy' snack foods.

In phase one DF rich products (at 5, 10 and 15 % w/w wheat flour replacement levels; total of 23 different samples) were incorporated into extruded snacks to determine the role of DFs in altering their physicochemical and nutritional characteristics. Starch digestion was shown to be lowered with all of high DF snacks ($P \leq 0.05$), however, this was not always dose responsive (oat bran and super gum showed no difference with increasing concentrations). Product texture and viscosity parameters were also affected by DF although no general pattern could be observed.

In phase two oat bran and psyllium material were incorporated into snack foods at 15 % (w/w) to evaluate potential GR *in vitro* and also *in vivo* (intervention study of 12 healthy subjects aged 18-40 yrs, with BMI 22.5-28, a total of 184 finger prick samples). Psyllium extruded snacks achieved attenuated *in vitro* and *in vivo* GR, ($P \leq 0.05$). Oat bran reduced the *in vitro* but not *in vivo* response ($P \leq 0.05$). Water absorption was negatively correlated with *in vitro* digestion (20 min) and *in vivo* AUC ($P \leq 0.05$).

In conclusion, the findings from this Ph. D. indicate the mechanism of DF ability to attenuate GR is related to its ability to bind water, and not all DFs behave in a similar fashion. Further research is required to elucidate the role of water in starch digestion and the impact on GR.

Declaration

None of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of Manchester Metropolitan University or any other university or institute of learning with the exception of Table 2.4. Table 2.4 was submitted in my M. Phil. thesis, *Dietary fibres and their properties:- the possibility of fibre lowering the glycaemic index of foods post extrusion*. Massey University, 2008.

List of publications derived from this project

Peer- reviewed journals

Brennan, M.A., Derbyshire, E.J., Tiwari, B.K., Brennan, C.S. (2012) Enrichment of extruded snack products with co-products from chestnut mushroom (*Agrocybe aegerita*) production: Interactions between dietary fibre, physicochemical characteristics and glycaemic load. *J. Agric. Food Chem.* **60**: 4396–4401

Brennan, M.A., Derbyshire, E., Brennan, C.S., Tiwari, B.K. (2012) Impact of dietary fibre-enriched ready to eat extruded snacks on the postprandial glycaemic response of non-diabetic patients. *Molecular Nutrition and Food Research.* **56**:834-847

Brennan C.S., Derbyshire, E., Tiwari, B.K., & Brennan, M.A. (2011) Effects Of Extrusion On The Polyphenols, Vitamins and antioxidant activity of foods. *Trends in Food Science and Technology* **22**:570-575

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Copies of which to be found in Appendix

Conference presentations:-

"The effect of fibre inclusion in extruded snack products on physical and textural properties of extrudates" RHISC Conference MMU July 2010

"The integration of beta-glucan fibre-rich fractions to form healthy extruded snacks" 5th International Dietary Fibre Conference Rome May 2012

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List of abbreviations

AACC	American Association of Cereal Chemists
AUC	Area Under the Curve
BMI	Body Mass index
cp	Centipoise
dB	Decibel
DEFRA	Department for the Environment and Rural Affairs
DF	Dietary Fibre
DNS	Di-Nitro Salicylic acid
DP	Degrees of Polymerisation
EU	European Union
FSA	Food Standards Agency
GI	Glycaemic Index
GL	Glycaemic Load
GR	Glycaemic Response
HDL	High Density Lipo-protein
IDF	Insoluble Dietary fibre
NSP	Non-Starch Polysaccharides
OAI	Oil Absorption Index
RDS	Rapidly Digested Starch
RTE	Ready to eat
RVA	Rapid Visco Analyser
SD	Standard Deviation
SDF	Soluble Dietary Fibre
SDS	Slowly Digested Starch
SEM	Standard Error of Means
SME	Specific Mechanical Energy
TDF	Total Dietary Fibre
WAI	Water Absorption Index
WHO	World Health Organisation
WSI	Water Solubility Index

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Chapter 1

Introduction, Project Rationale

1.1 Introduction

The current study was derived from results and experiences from my previous studies for my award of an MPhil at Massey University, New Zealand. The research concentrated on the role of a number of dietary fibres in modifying the rate of starch degradation in extruded snacks (Brennan *et al.*, 2008a,b). Reflecting on the results obtained, and the increasing amount of literature regarding the link between novel and traditional dietary fibres and human health, a research proposal was derived to determine the effect of a range of these traditional and non-commercial (derived from waste streams of the food industry) forms of dietary fibres and how they relate to both potential *in vitro* and actual *in vivo* glucose responses. This chapter gives the full background to the present study, the aims of the research and the structure of the whole thesis.

1.2 Background to study

Food processing and retail production have a significant impact on the environment in terms of the amount of food waste material sent to refuse facilities in the UK every year (approximately 800,000 tonnes according to DEFRA, (2010)). Although much of this waste is through inefficient utilisation of processed foods (i.e. ineffective control of sell by date material and over catering in restaurants), a substantial amount arises from the trimming activities of food processing and manufacture. Components derived from food waste streams have been reported to be high in dietary fibre (DF) and phytochemicals and may have beneficial nutritional effects when incorporated into food products (Stojceska & Ainsworth 2008). A number of researchers have investigated the possibility of using waste derived from the food waste stream to produce food commodities. For instance Dehghan-Shoar *et al.*, (2010; 2011a,b) have successfully incorporated fibre and bioactive ingredients from tomato waste products to produce lycopene rich extruded snacks. Similarly, Norfezah *et al.*, (2011) have used waste material derived from pumpkin processing to produce fibre rich extruded snack products. It is logical, therefore, to imagine that co-product recovery from food waste streams is a real possibility and that the better utilisation of fibre rich waste food materials will lead to significant environmental and financial benefits for the sustainable food industry.

Consumers now have a greater awareness of the need to eat healthier high fibre foods. Despite this attention on functional foods, fibre-rich foods and healthy eating, there is a growing preference by the consumer for ready to eat (RTE) food products for quick and easy consumption to accompany changes in lifestyles (Euromonitor, 2011). It would appear that the pressures of a demanding lifestyle can outweigh the nutritional judgements regarding a healthy diet. Many of the RTE products available to the consumer on the supermarket shelves are in the form of extruded snack products. Paradoxically from a nutritional viewpoint, extruded snack products are traditionally manufactured from highly refined flours of grains such as wheat, maize and rice. These foods are generally energy dense, low in fibre and phytochemicals and are considered as high glycaemic index food products, due to the fact that the shear developed within the extruder during extrusion processing increases the rapidly digestible carbohydrate composition of the food product, all of which ultimately impact on health when considering glycaemic response and diabetes (Brennan, 2005).

Extrusion is a continuous cooking process that under the appropriate conditions forms a product with improved nutritional, textural, sensory qualities that appeal to the modern day consumer in that the expansion of materials due to extrusion creates highly porous and crispy products (Hardacre *et al.*, 2006; Dehghan-Shoar *et al.*, 2011a,b; Norfezah *et al.*, 2011). Previous studies regarding the inclusion of waste material derived from cauliflower and spent brewers grains have shown that these materials can negatively impact on product taste, imparting off-flavours, as well as making products less acceptable in appearance to consumers (Stojceska *et al.*, 2008).

Recent studies have identified that the nutritional quality of extruded cereal foods can be improved by incorporating material obtained from conventional food waste systems to give effects similar to incorporating whole grains and different forms of DFs (Brennan *et al.*, 2008a,b; Stojceska & Ainsworth, 2008; Stojceska *et al.*, 2008; Sobata & Redzedzicki, 2009; Dehghan-Shoar *et al.*, 2011a). This two-fold approach to increasing fibre intake and plant phytochemicals has been shown to lower the glycaemic response of cereal foods (mainly through delayed gastric emptying) and also colon cancer (through the promotion of fermentation action of microflora increasing levels of butyrate within the small intestine) (Brennan, 2005). At the same time, an increase in β -glucan content of the diet has been correlated to a lowering of HDL cholesterol levels within healthy individuals. Similar benefits in terms of regulation of weight and cholesterol levels as well as increased ability to manipulate cancer prevention (most notably colon cancer) have been associated

with whole grain intake (Koh-Banerjee & Rimm, 2003; Hiller *et al.*, 2011; Poutanen, 2010; Hlebowicz *et al.*, 2011).

1.3 Ph. D. aims

The aims of this Ph. D. are to utilise material high in fibre and phytochemicals derived from food processing waste streams, as well as conventional fibre rich fractions, into RTE snack products, so as to improve the nutritional quality of such food products. Specifically to:-

1. Evaluate suitable commercially available DF and DF in the co-products of food waste stream production for inclusion into fibre rich extruded snack products.
2. Analyse and determine the relationship between the incorporation of fibre-rich materials into extruded food products and their effects on the physicochemical properties of the food products and sensory analysis characteristics.
3. Evaluate the effect of extrusion technology on the nutritional composition of the extruded snack products (namely starch digestibility and glucose release).
4. Compare and contrast the glycaemic response of fibre rich products recorded using *in vivo* and *in vitro* analysis.

1.4 Thesis outline

The thesis is divided into nine main chapters. *Chapter one* introduces the readers to the general topic and the aims of the thesis. *Chapter two* presents a detailed literature review of the subject so as to illustrate the historical and current perspectives of the issues. *Chapters three, four and five* describe the materials and methods used for all of the experimentation whilst conducting the research work into this area. The results of the work are then divided into two chapters for ease of presentation. *Chapter six* describes the results obtained in the initial screening of different dietary fibres, potential waste stream processing material and potential material from grain based products. This evaluates the physicochemical and nutritional properties of snacks derived from a total of eight fibre rich sources at different levels of inclusion. The results from the product characteristics and *in vitro* digestibility evaluations of the 23 extruded products illustrated in this chapter were used to make an informed decision as to two products to evaluate in terms of *in vivo* glucose response. *Chapter seven* details the product characteristics, *in vitro* and *in vivo* nutritional quality of

three test extruded snack products based on the observations of *Chapter five*. *Chapter eight* forms the major discussion chapter of the thesis where the results of *Chapter six and eight* are discussed and evaluated in detail. *Chapter nine* represents the overall conclusion from the research and future directions for research. At the end of the thesis there is a comprehensive list of references together with an appendix to include data, information sheets and consent forms appertaining to elements of the research.

Chapter 2

Literature Review

2.1 Introduction

This chapter reviews the research which has been conducted over the last century with regards to the role of fibre in food products (especially RTE extruded food products) and the effect of fibres on the modulation of glucose release from food products. The basic principles of extrusion technology are also reviewed to explain the relationship between extrusion processing and starch digestibility, as well as a section relating to both *in vitro* and *in vivo* analytical procedures used in the evaluation of glucose release from foods.

2.2 Ready to eat products

2.2.1 Definition of ready to eat products

Ready to eat foods are increasingly popular with the consumer predominately due to their convenience of consumption and ease of preparation and storage. In essence RTE food products are those which require minimal (if any) preparation prior to consumption. Consumer interest in RTE snack food is growing. From an early date it has been assumed that this demand was due to factors such as convenience, value, attractive appearance and texture (Harper, 1981).

The Food Standards Agency (FSA, UK) defines ready to eat products as:

“any food for consumption without further heating or processing”. This definition covers both open and pre-wrapped ready to eat products and is intended to apply whether the ready to eat food may be consumed hot or cold. The expression ‘further heating or processing’ is not intended to include food preparation activities such as light washing, slicing, chopping, portioning, marinating or preservation carried out by the consumer by way of preference to an otherwise ready to eat food item.” (FSA, 2011).

Under this definition a number of processed foods can be regarded as RTE products including biscuits, crisp, breads, pies, sandwiches and rolls, dairy products (milk, cheese, spreads), prepared salads and vegetables, and fruit. The list can be extremely long and with new products entering the food market nearly every day the list is getting longer and longer (Fast, 1999).

One important sector of the RTE product market is the cereal RTE segment. This is traditionally dominated by extruded snack products, for instance breakfast cereals, extruded cereal shapes, and cereal biscuits / bars. These products originate from the rise of the breakfast cereal market in the United States of America in the late eighteenth century and the beginning of the twentieth century, and the development by the Kellogg brothers of healthy vegetarian foods related to human nutrition (Fast, 1999). Originally the majority of these products were derived from whole grain sources and were predominately flaked from steamed grains (the steam making the grains pliable to be reformed by the flaking process).

The popularity of flaked cereal products exists in terms of corn flakes or bran flakes which are the mainstay of the product range for many cereal food producers (Personal communication, B. Fast AACC professional development course Switzerland 2010). However with the advent of more intense flour separation techniques and refining processing, partly driven by the consumer expectations of white food products and products with a finer taste and texture, more recent extruded cereal snacks are derived from mixtures of flour components rather than being whole grain in nature. This has an obvious impact on the composition of the raw material and hence the nutritional quality of the product. The milling and refining of cereals concentrates the amount of starch in a food product, removing the outer coats of the cereal grain (Seal, 2007). As the outer coat of the cereal grain is rich in fibre and phytochemicals, modern refined white cereal flours are high in starch material but low in bioactive ingredients (such as ferulic acid and arabinoxylan) associated with the cereal grain outer layer (bran). To counter this negative impact on nutrition recent research has been conducted to investigate the possibility of utilising whole grains or whole grain components in RTE foods (Poutanen, 2010).

2.2.2 Definition of snack foods

As mentioned previously, RTE food products are convenient items which appeal to the consumer eating habits (Fast, 1999). An important segment of RTE market is the snack product. As with the RTE food sector, the snack food market is expanding rapidly and continued growth is forecast into the future. Market size of snack foods in the UK is expected to reach £4,600 million in 2011 with production volume of 448,000 tonnes (Euromonitor, 2011). There is a large range of snack foods on the supermarket shelves with a large variety of sizes, shapes, colours and flavours available designed to attract the consumer.

Of this market, potato crisps/chips generally dominate the snack food market followed by corn (maize) chips. Most snacks are made from starch based products (e.g. corn, wheat, rice, oats and potato). These products are usually high in starch content but low in nutritional value in terms of vitamins, minor minerals, amino acids and fibre (**Table 2.1**).

During the last ten to fifteen years consumers have become more health conscious and are demanding snack products which are healthier and more nutritious than those previously available (Euromonitor, 2011). Thus the consumer is demanding a convenient, RTE product which satisfies their hunger requirement and yet is low fat, rich in DF, and is potentially fortified with vitamin and minerals.

Table 2.1. Composition of ready to eat snacks.

Ready to Eat Snacks	Starch (g/100 g)	Total sugars (g/100 g)	Dietary fibre (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Water (g/100 g)	Energy values (Kcal/100 g)
Popcorn	15.5	62.1	n/a	20.0	2.1	2.6	480
Potato	52.6	0.7	5.3	34.2	6.2	2.8	530
Tortilla	58.9	1.2	6.0	22.6	7.6	0.9	459
Breadstick	67.5	5.0	3.8	8.4	11.2	3.5	392
Cereal bar	28.3	27.6	4.8	22.2	10.4	2.6	468
Kit kat	12.9	50.1	1.4	26.0	7.5	2.0	500
Crispbread	67.4	3.2	11.7	0.6	9.4	6.4	308
Rice	82.5	10.4	0.7	1.0	6.1	3.0	382
Corn	81.4	8.2	0.6	0.9	7.9	3.0	376

Adapted from (McCance & Widdowson 2002).

This move towards healthier snack products is driving manufacturers away from the sugar and starch rich foods of traditional snack products into a balanced formulation of potentially added value ingredients in a nutrient rich but energy low food product. This in itself presents numerous problems associated with ingredient formulation and processing parameters, such as increased hardness, reduced expansion and lowered organoleptic quality.

Such a healthier snack food could be produced by the incorporation of naturally derived phytochemical ingredients (from fruits and vegetables) so as to increase the amount of fibre and micro-nutrients into the food product (Altan *et al.*, 2008; Vitaglione *et al.*, 2008).

2.3 Snack consumption, consumer grazing and satiety

Recent research attention has focussed on the link between RTE snacks, snacking and nutritional impact. For instance there has been a long standing relationship between snack intake and skipping of meals or grazing during the day (de Graaf, 2006). Early research has indicated that the skipping of meals has a negative effect on mood and behaviour (Lindeman & Clancy, 1990). Wolfe *et al.*, (1994) also suggested a relationship between obesity levels in children and their incidence of breakfast skipping.

2.4 Extrusion technology as a vehicle for snack production

In 1797, Joseph Bramah patented the first extrusion process for making lead pipe. For the last 250 years extrusion technology has been used in the manufacture of plastics, moulded metals and synthetic materials. It is only relatively recently (since the 1970's) that there has been an appreciable use of extrusion technology in the food industry. The interest of the use of extruders in the food industry stems from the fact that they are capable of blending diverse ingredients into novel food structures and hence may be useful in the development of functional foods. The quality of the final products may vary and depend on a few variables of extrusion parameters such as raw materials composition, feed moisture, barrel temperature, screw speed, type of extruder and screw configuration (Miller & Mulvaney, 2000).

The major ingredients of most snack foods in the market are corn, wheat, rice, potato and oats based. Numerous papers have discussed the role of extrusion technology in the manufacture of wheat and corn products (Unlu & Faller, 1998; Holguín-Acuña *et al.*, 2008; Sobata *et al.*, 2010). Recent research has focussed on the utilisation of DF/non-starch polysaccharides (NSP) in the formulation of extruded snack products (Brennan *et al.*, 2008a,b; Parada *et al.*, 2011). The incorporation of fibre in the extruded snack generally results in changes of extrusion parameters (torque, SME, pressure thrust and energy consumption), chemical properties (nutrient profile), and physical properties (structure and texture) of the final extruded products. However, there is still a dearth of information and knowledge on how the different types of fibres affect the mixture properties and their behaviour in an extruder system.

Ingredients and formulation play an important role towards final quality texture of an extruded snack product and may have a major effect on the consumer acceptability and functional properties of the extruded product.

Extrusion is a process that involves mixing, forming, texturising and cooking raw material into a food product. At the start of the extrusion process raw material is placed into the extruder barrel at relatively high moisture content (between 8-16 %) whereas the moisture content of the final extruded products generally are 4-6 % (Sumathi *et al.*, 2007). Thus the extruder can act as a dryer reducing the moisture content of raw materials during the cooking of a product. The reason for this moisture loss is the requirement of releasing pressure after passing through the extruder, this pressure release has to be achieved quickly and as a result excess moisture is flashed off as steam. The process of flashing off moisture and pressure equalisation of the relative fluid product in the extruder causes rapid expansion and inflation of the product post extruder die and in itself generates a puffed texture with numerous expanded gas cells.

The structure and configuration of this puffed product is important in terms of understanding the consumer quality of the product (Kim *et al.*, 2009). High expansion ratio (as measured by the percentage increase in product diameter post extrusion, compared to the die size) equates to increased porosity of the product and either a large number of gas cells, or a number of large gas cells. Generally the extruded product has a relatively hard outer coat, representing a layer of collapsed gas cells, and an expanded inner matrix composing of these gas cells. The texture of the extrudate is dependent on both the extent of expansion (related to the amount of gas cell space in the extrudate) as well as the integrity of the outer coat of the extrudate. The outer coat of the extrudate is generally important in terms of moisture penetration and is essential to consider when producing breakfast cereals, with sufficient bowl life for the consumer, or RTE snacks, which need to remain crisp for a long storage time. The internal structure is also important in terms of the perceived crispiness of the product. A connected network of fine gas cells is produced from homogenous raw material and represents a consistent crisp product. Extrudates that are formed from non-homogenous material may have a number of large gas cells, connected by smaller gas cells, this can lead to a brittle material which fractures relatively easily (Pai *et al.*, 2009; Burtea, 2001). Whilst the consumer would wish to have a crispy, expanded product, there is a fine balance in terms of brittleness and crispiness when it comes to packing quality and the potential brittleness of a product causing broken shapes

during storage. Generally highly expanded extruded products are less dense than unexpanded products.

Different raw materials with different functionality may provide the effect on the formation and stabilisation of the final product quality such as colour, flavour and nutritional quality (Guy, 2001a). In addition, different ingredients affect the rate and degree of expansion of a product as it exits the extruder die, and hence can modulate the texture of the extruded product. Sugars, lipids, salts and fibres (Guy, 2001a; Robin *et al.*, 2011a) all affect the physical properties of extruded products. For instance sugars and lipids can lead to a reduction of extrudate viscosity within the extruder barrel, this in turn can reduce the amount of shear and pressure (and overall SME) in the extruder and hence a reduction in the amount of pressure required to be released at the die face. The reduction in pressure difference between behind and after the die can result in a poorly expanded product. In addition, salt and fibre ingredients may reduce the amount of available (free) water in the extrudate in the extruder barrel. This may result in an increased amount of pressure in the system, however the water may be less available for flashing off at the die face (being held more closely in combination with these ingredients) and consequently produce an unexpanded product. In this case there should therefore be a relationship between high moisture loss of the extruded product (moisture of raw material in barrel - moisture of final product) and expansion and crispiness.

2.4.1 Extrusion processing and its impact on food quality

Extrusion cooking is one of the most important food processing technologies which has been used since the mid 1930's for the production of breakfast cereals, RTE snack foods, and other textured foods (Burtea, 2001). Over the years, extrusion cooking has become a major processing method for food and feed industries, such that since the 1970's researchers have turned this rapidly evolving process from an art into a science (Riaz *et al.*, 2009). Extrusion cooking has been studied extensively to produce variety of specialty foods including pasta products and RTE breakfast cereals, baby foods, snack foods, texturised vegetable protein, pet foods, dried soups and dry beverage mixes. Extrusion not only improves digestibility of both protein and starch (Singh *et al.*, 2010) but also improves the bioavailability/bioaccessibility of nutrients (Gu *et al.*, 2008; Brennan *et al.*, 2011; Dehghan-Shoar *et al.*, 2011a) compared to conventional cooking. In addition to these properties, extrusion cooking is preferred over conventional cooking/processing techniques because of its ability to develop a range of products with distinct textural

advantages including expansion, crispiness and general organoleptic qualities; being versatile, high productivity, low operating costs, energy efficiency and shorter cooking times.

In essence, extrusion can be regarded as a continuous cooking process, however this interpretation is relatively simplistic. There are three major screw-types: single-screw and intermeshing twin-screw in either counter rotating or co-rotating styles (Miller & Mulvaney, 2000). Whichever screw-type is used it constantly rotates within the barrel during extrusion, thereby propelling food material forward creating continuous pressure and shear. At the end of the screw the product is forced (at high pressure and temperature) through a restrictive orifice commonly called the die. For continuous cooking the single-screw type is most commonly used as it is simple to use and costs less. The ingredients are transported via shear and as a consequence there is an amount of back mixing (Janssen, 1989). The intermeshing twin-screw extruders have a variable residence time (time spent travelling along the barrel before leaving the extruder die) of the product along the barrel so that as the product travels along the barrel it generally experiences a positive displacement pumping mechanism effect (Janssen, 1989). In both cases (twin and single screw) the geometry of the barrel, screw and die have similar effects on the mechanical energy applied to the product during the extrusion process and hence the amount of mixing and cooking the product is subjected to during its residence time in the barrel.

As the product passes through assembly at the end of the screw-barrel system it melts in the die due to the high pressure (above the vapour pressure of water) and high temperature (above 100 °C). The flash pressure reduction at the interface between the die face and the atmosphere, with the resulting evaporation of the water present, means that the molten product behind the die face then expands considerably at the die face. The rate of expansion depends on the rheological and thermal properties of the molten material and on the geometry of the shaping insert (Guy, 2001a). The cutting of the expanded extrudate is usually done by a rotating knife on the outer face of the die. The pieces, also known as collets, can be coated with sugar, flavour or coloured molasses (Burns *et al.*, 2000).

Extrusion cooking includes a wide range of products, pet food, expanded snacks, breakfast cereals, pastas and infant foods. The rheology of the pastes within the extruder has a significant influence of the product characteristics (bubble growth rate, degree of expansion and curvature). Differences in flow rate and hence product characteristics can be created by changing the process parameters of die pressure drop, screw flow dynamics or

screw energy consumption. **Figure 2.1** shows different factors that have an influence on the extrusion cooking process.

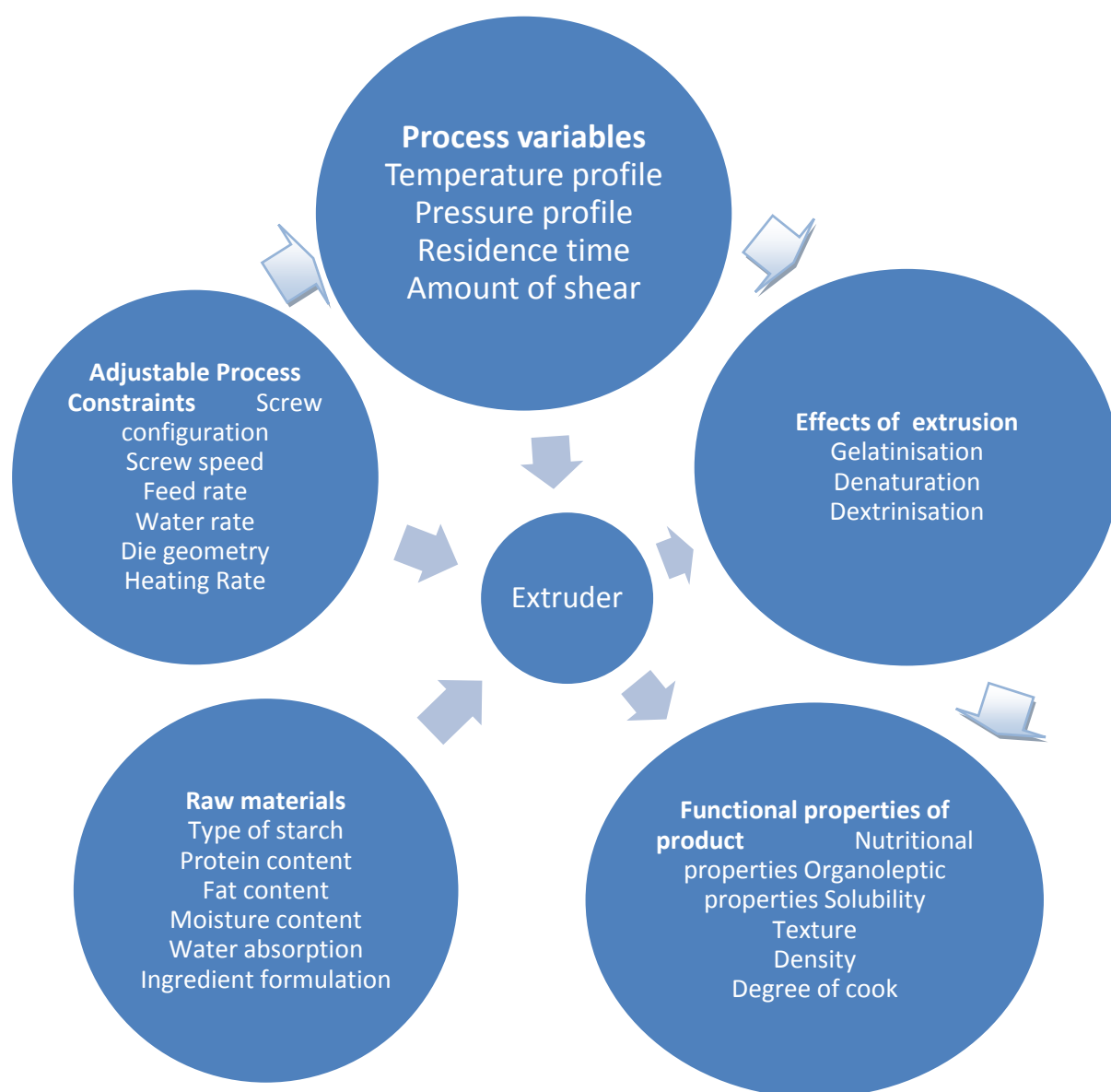


Figure 2.1. Interaction of raw materials, process variables and extruder to form product.

2.5 Nutritional properties of extruded snacks, as affected by extruder cook and shear

Humans cannot easily digest ungelatinised (raw) starch. Although the extrusion process can “cook” starch, complete gelatinisation may not occur but digestibility will be improved due to partial gelatinisation and fragmentation of starch. Extrusion can be regarded as a pre-digestion of starch, potentially similar to the action of yeast fermentation in bread making, as the branches of amylopectin are susceptible to shear forces and can be cleaved

from the main chain causing a change in the functionality of the ingredient. The depolymerisation of the starch, combined with the high temperatures associated with extrusion, contribute to the fact that starch will be made more readily available to amylolytic enzymes during digestion, and hence extruded snack products tend to yield a higher glycaemic response compared to their unprocessed raw ingredients (Brennan *et al.*, 2008a). Both amylose and amylopectin have been found to exhibit a reduced molecular weight distribution following extrusion processing (Politz *et al.*, 1994a). In a similar manner it has also been documented that extrusion of wheat starch can be optimised to maintain specific molecular weights and produce defined starch products (Politz *et al.*, 1994b).

However, research has also suggested that these shorter branches could cross link forming novel, indigestible linkages and therefore lower the glycaemic index (Theander & Westerlund, 1987). These complexes may be regarded as resistant starch material and potentially contribute to the overall DF content of the food product. High amylose rice has been extruded into noodles which then have a reduced glycaemic index (Panlasigui *et al.*, 1992). This ability to modify the molecular structure and hence functionality of starch is of obvious use to the food industry not only for when trying to derive products with known digestibility properties but also when trying to produce novel structures and textures which will appeal to the sensory expectations of the modern consumer.

2.6 Waste stream products in the food industry

2.6.1 Definition of waste stream

The European Union defined waste in 2006 as ‘any substance or object ... which the holder discards or intends or is required to discard’ (Directive 2006/12/EC). At the same time the EU also required that ‘the recovery of waste and the use of recovered materials as raw materials should be encouraged in order to conserve natural resources (Directive 2006/12/EC). As a result of this a waste hierarchy has been developed (DEFRA, 2007).

As can be seen in the diagrammatic representation (**Figure 2.2**) waste prevention is considered to be the most environmentally effective solution. Re-using products and materials for the same or a different purpose would be the next tier in waste reduction. Recycling and composting come lower down the hierarchy. Energy recovery is the final consideration before substances should be disposed of as waste i.e. they have no further use.

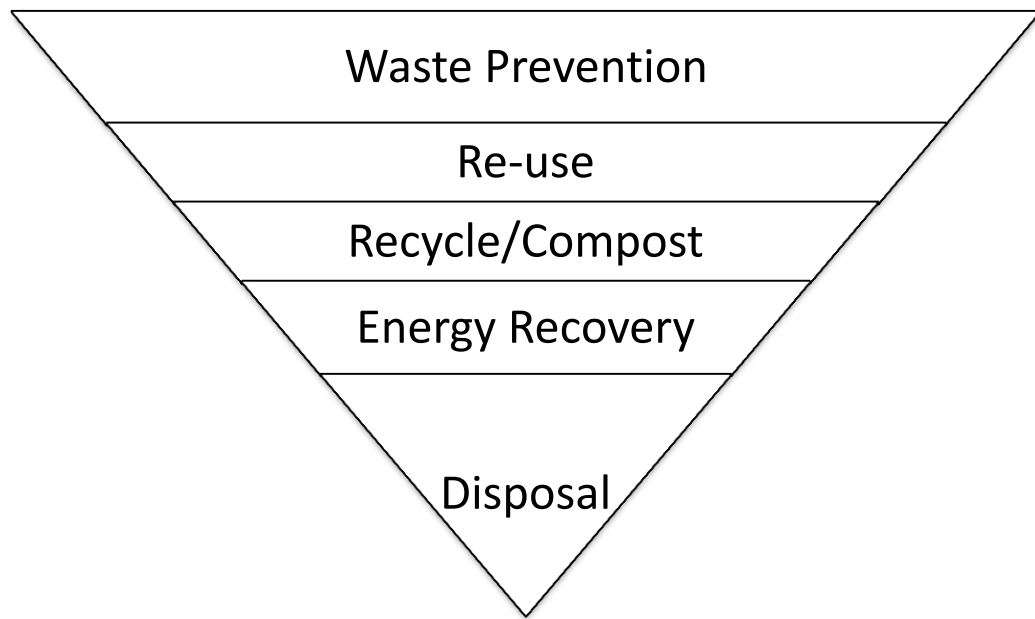


Figure 2.2. The waste hierarchy (Source DEFRA, 2007)

The overall objective for waste policy is to improve the future potential of the environment and also of human health by producing less waste. There is also a possibility that by utilising waste streams as a resource wherever possible then it is possible to develop sustainable supply chains for the food industry. Through more sustainable waste management – reduction, re-use, recycling, composting and using waste as a source of energy – the government aims to decrease the environmental impact of waste.

2.6.2 Sources of waste products from food production and their potential use as added food systems.

There are many sources of food waste in the food industry today. These include material from the trimming and preparation of vegetables before leaving the farm gate, additional trimming from the preparation of material at processing plants, material used in the preparation of food products but not included in the final product, waste material derived from non-consumption or non-use of processed foods. For instance in the processing of vegetable soup there are several potential waste streams starting from the trimming of excess foliage or stems from the farm and the rejection of misshapen or unusable vegetative material by the processor. During the processing of the soup there may be waste derived from the removal of seeds or skins from the vegetable material, and after processing there may be waste in the form of under or over processed material deemed unsuitable for consumer use.

Table 2.2 illustrates the amount of food waste which is generated in such a way and the potential ways to dispose of this waste. Researchers have investigated ways in which to better utilise the 604,883 Tonnes of waste generated each year. For instance Norfezah *et al.*, (2011) have used pumpkin waste from growers and processors of this vegetable in food products and have found wastes from the seeds and also the skins (outer peelings) of pumpk in can be a high source of fibre and protein. The recovered fibre components have been utilised in food systems to produce added fibre foods which have shown potential in the regulation of blood glucose (Norfezah *et al.*, 2011).

Similarly Dehghan-shoar *et al.*, (2010; 2011a) have used waste material from the tomato processing industry in the form of tomato peel from tomato paste production. The researchers have characterised these materials and identified that these waste streams are rich in lycopene. Further experiments have shown that the lycopene components can be incorporated into extruded snacks to produce novel foods rich in bioavailable lycopene (Dehghan-Shoar *et al.*, 2011b).

2.6.3 Potential under-utilised waste food stream products

To date no research has been conducted on the recovery and utilisation of bioactive ingredients from mushroom growing. The cultivation of mushrooms creates a substantial amount of waste material in the form of stalks and base hyphae material left behind after the cutting of mushroom caps. A conservative estimate is that only 40 % of the mushroom biomass is harvested during cultivation, the remaining 60 % of mushroom biomass is left behind to be disposed of as compost material (personal communication Oakland Farms, Evesham 2009). However, some of this substantial amount of material may be useful as a source rich in fibre (β -glucan) and protein. There is therefore a significant reason for the investigation of the potential use of this waste material in food systems.

Additionally, the milling industry is another major source of waste products for the food industry. Milling of oats and barley creates a waste stream of bran and associated husk material. Whilst the food industry utilises a small amount of bran material for bulking substances to cereal and meat products, about 80% of the milled bran is sold to the animal feed industry at a low economic return to the miller. Recent research has focussed on the utilisation of bran as a source of added fibre components and bioactive co-passengers (Laerke 2011). It makes sense in terms of sustainable food production, to fully utilise these waste streams in mainstream food production.

Table 2.2. Disposal and recovery routes for each waste type according to Food and Drink Federation member site returns

Waste hierarchy	Recovery & disposal options	Food Waste^a T	Packaging Waste^b T	Mixed food & Packaging waste^c T	Total T
Recycle/ compost	Anaerobic digestion	66,239	0	0	66,239
	Composting	34,607	3,120	2,599	40,326
	Recycling	162,633	58,556	7,315	228,504
Recovery	Land spreading^d	216,345	0	1,980	218,325
	Thermal treatment^e	93,975	296	0	94,271
	Other recovery	5,392	21,352	11,805	38,549
Disposal (lowest)	Incineration without energy recovery	4,037	1,065	1,424	6,526
	Landfill	17,569	10,511	109,686	137,766
	Other disposal	4,086	0	10	4096
	TOTAL	604,883	94,900	134,819	834,602

Notes: a Total **food waste** arising which left via the backdoor of the factory in 2006, unmixed. It includes any inedible fraction, possibly also some materials considered as by-products utilised for example in animal feed or human food but not food waste mixed with packaging waste.

b Total **packaging waste** arising which left the factory via the backdoor in 2006, unmixed. It does not include reusable packaging unless it had reached the end of its life nor any packaging mixed in with food waste.

c Total **mixed food and packaging waste** arising, i.e. finished goods or food and packaging waste which arose separately but was mixed on site before leaving via the factory backdoor, e.g. in a single skip.

d **Liquid wastes and sludges** that were landspread or tankered overland to a sewage treatment plant. It does not include trade effluent transferred via public sewer to a municipal waste water treatment plant.

e **Thermal treatment** includes traditional mass burn, as well as alternative processes which involve energy recovery. Adapted from (Bartlett *et al.*, 2008).

2.7 Dietary fibres and their use in the food industry

2.7.1 The definition of dietary fibre

Historically the term “dietary fibre” (DF) has been used to define a collection of plant based cell wall materials, with Hipsley (1953) first using the term to describe plant cell wall material which he proposed was protective against pregnancy toxemia. Trowell renewed usage of the term in 1972 describing it as the “skeletal remains of plant cells resistant to hydrolysis by the enzymes of man” and “synonymous with unavailable carbohydrate” (Trowell, 1972a,b). Eventually the definition was broadened to include all digestion resistant polysaccharides (Trowell *et al.*, 1976), although mostly plant storage saccharides such as gums, pectins, oligosaccharides, mucilages and modified celluloses, it also included cellulose, hemicelluloses, lignins and their closely associated substances such as waxes, cutin and suberin. More recently there has been an attempt to improve the specificity of terms, so that the following definition was developed by a committee of members of the American Association of Cereal Chemists (AACC).

"Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human intestine with complete or partial fermentation in the large intestine. DF includes polysaccharides, oligosaccharides, lignin, and associated plant substances. DF promotes beneficial physiological effects, such as laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (AACC, 1999).

This definition had been in use for a decade when an EU Commission Directive (2008/100/EC) clarified the definition of dietary fibre:-

“For the purpose of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed;
- edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic, or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
- edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.”

This definition links the chemical composition of fibre to its physiological effects. At the same time it includes all non-starch polysaccharides resistant to digestion in the small

intestine and fermentable in the large intestine (celluloses, hemicelluloses, pectins, modified celluloses, oligosaccharides and polyfructans such as inulin, gums and mucilages). It also includes oligosaccharides and polysaccharide components bound to the plant cell wall (lignin, waxes, cutin, and suberin). Materials with analogous characteristics to DF are included when they “have a beneficial physiological effect.”.

A non-exhaustive list of the constituents of DF can be summarised as in **Figure 2.3** to relate to actual plant components and physiological effect.



Figure 2.3. Constsituents of dietary fibre.

2.7.2 Physicochemical properties of dietary fibre

Until relatively recently DF used to be considered as an inert carbohydrate fraction with little nutritional value, however current research has shown it is an essential component of

our diet. For instance the consumption of foods rich in DF has been associated with decreased risks of developing diet related chronic diseases represented diagrammatically in **Figure 2.4** and their physiological effects are usually compared with the intakes or contents of total dietary fibre (TDF) (World Health Organisation (WHO), 2003). The use of the term TDF is a remnant from our appreciation of fibre as crude fibre by animal nutritionists. In reality DF now refers to a large number of substances encompassing very diverse polysaccharide macromolecules, which also exhibit a large variety of physicochemical properties and various degrees of hydration (or solubility).

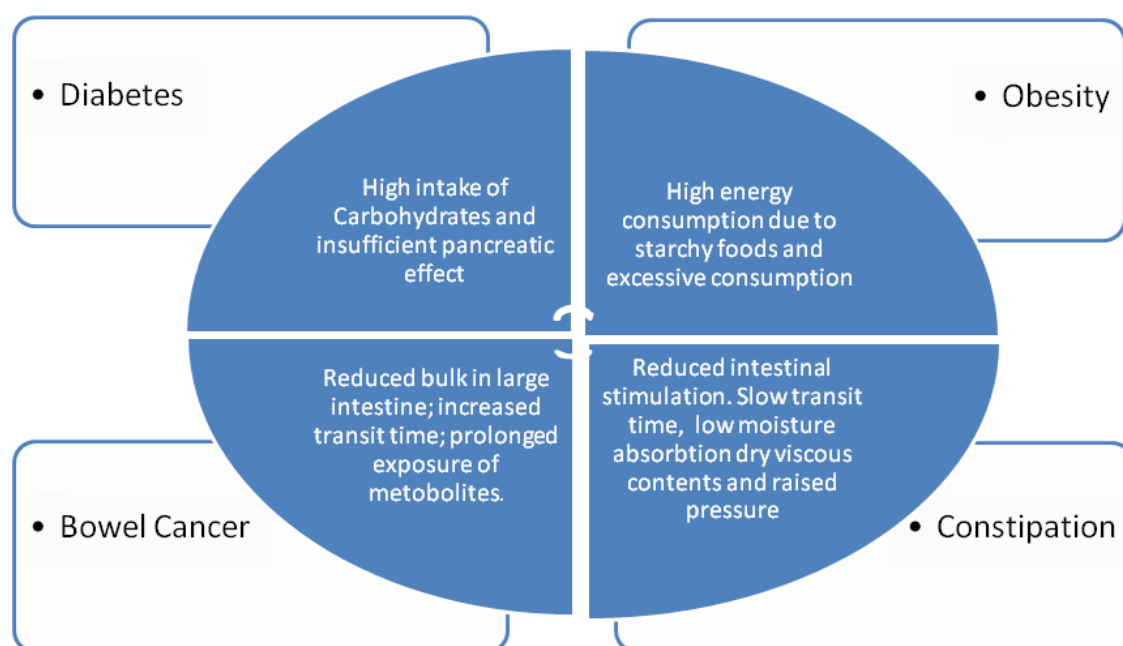


Figure 2.4. How a lack of dietary fibre influences human physiology.

As a result of compositional variations, different sources of DF have different metabolic and physiological effects depending upon the chemical and physical properties of the DF (Topping, 2007; Lattimer & Haub, 2010). **Figure 2.5** gives a representation of the physiological effects of DF on the human body and illustrates the complexity of the interactions. An understanding of these contributory factors is useful in predicting the physiological response to a source of fibre and is outlined in the model proposed in **Figure 2.5**. Thus DF can be classified either according to their chemical structure (origin) or their ability to combine with water (solubility).

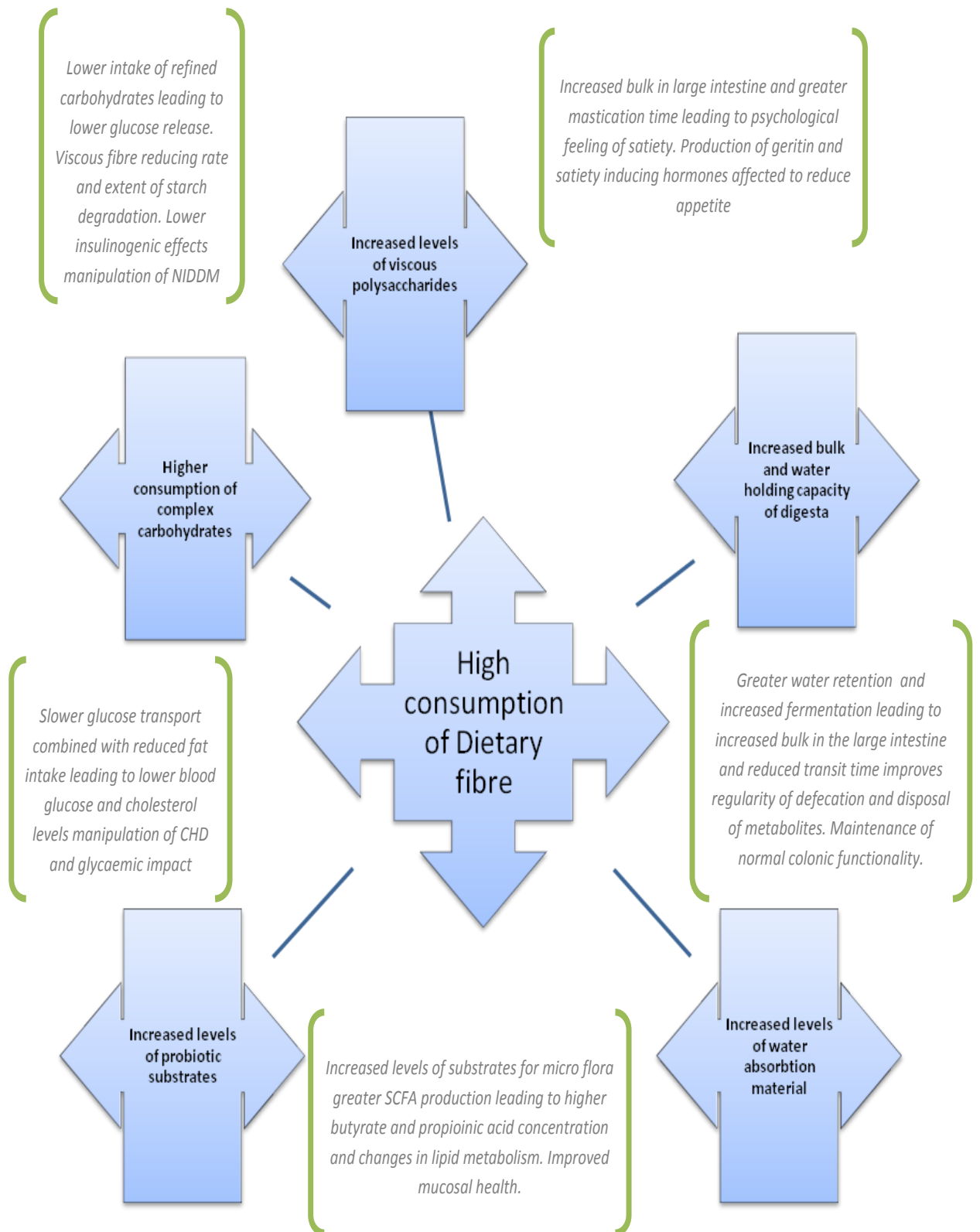


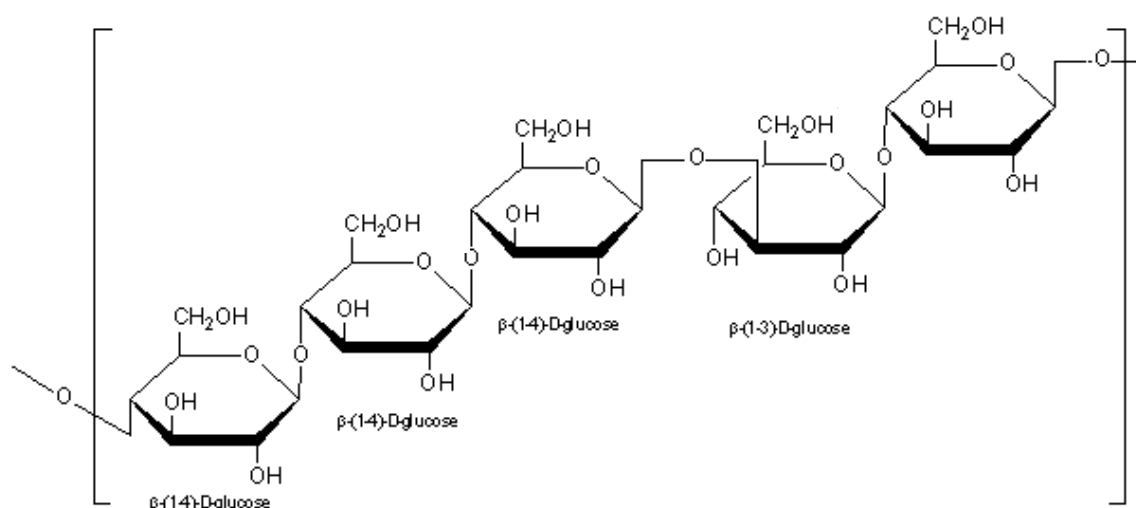
Figure 2.5. Physiological effects of dietary fibre on the human body
 (Adapted from Southgate 1990)

2.7.3 Structural aspects of dietary fibre

Dietary fibre includes primarily polysaccharides along with oligosaccharides, and substances from plant cell walls associated with the non-starch polysaccharides as noted in **Figure 2.3**. The classification of DF is a nutritional categorisation, not an exact description of a dietary component, however the commonality that DFs have is that they are not digested in the small intestine. On entering the large intestine many of them undergo complete or partial fermentation; their effects on metabolism and disease risk, therefore, are likely to be mediated through their properties as they pass through the gastrointestinal tract.

As previously mentioned DF consists mostly of polysaccharides and oligosaccharides. Oligosaccharides and polysaccharide molecules are composed of glycosyl units in linear or branched arrangements. The degree of polymerisation (DP) for oligosaccharides is 3-9 and polysaccharides 10+ (Nantel, 1999). Polysaccharides are characterised by their monosaccharide unit(s), and the nature of the linkages between them (Clark, 1992).

The simplest structure is that of homoglycans, where all the glycosyl (monosaccharide) units are the same; for example, cellulose and β -glucan both utilise the monosaccharide glucose. In cellulose the linkages are β (1-4) and the structure is linear, whereas in β -glucan, β (1-4) linkages are interspersed with (1-3) linkages resulting in a kinked structure (**Figure 2.6**). These materials are commonly found in oat and barley grains and have been related to the potential health benefits of ingestion of these grains.



<http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/carbohydrate-analysis.html>

Figure 2.6. Diagrammatic representation of barley β -glucan

Where there are differing monosaccharide units these are known as heteroglycans, for example hemicelluloses, with a back bone of xylan, galactan or mannan and side chains of

arabinose or galactose (arabinoxylans). Pectins are also heteroglycans with a galacturonic acid core being esterified to a varying extent with methoxyl groups (Clark 1992).

The behaviour of a polysaccharide in combinations with other ingredients is dominated by its physical properties. This in turn is a result of the structure and conformation of its linkages (sometimes described as ordered or disordered 'random coil' chain geometry) (Selvendran, 1984; Light, 1990). These chain conformations and chemical structure not only affect their physiological role as nutrients such as fermentability (Cummings 1973; Dikeman & Fahey 2006; Roberfroid 2007) but can regulate its hydration properties, solubility/dispersability in water, rheological properties, and ability to adsorb or bind bile acids (Tudorica *et al.*, 2002; Chaplin, 2003).

2.7.4 Hydration properties of dietary fibres

Many polysaccharides are comprised of monosaccharide chains, containing glycosyl residues which in turn have hydroxyl groups that are free to create hydrogen bonds with water molecules. It is hardly surprising then that glycans exhibit a strong affinity for water with their hydrophilic molecules hydrating readily when water is available. Polysaccharides in an aqueous system, such as the digestive system, will take up water, swell and either completely or partially dissolve.

The different chemical structure of DFs will create individual hydration characteristics for example swelling values range from 5.65 ml/g for resistant starch (Novelose) to 10.45 ml/g for citrus fibre, and water retention capacity range from 2.95 g/g for Novelose to 10.66 g/g for citrus fibre (Robertson *et al.*, 2000). The manufacturing processes of grinding, drying, heating and extrusion will alter hydration properties of polysaccharides due to the impact they have on the physical properties of the fibre matrix (Guillon & Champ 2000). Hydration characteristics for a variety of DFs are presented in **Table 2.3**.

Many researchers have investigated the physiological effects of DF in relation to hydration characteristics and the subsequent technological aspects important in their use in modern food technology applications (Guillon & Champ 2000; Chaplin 2003). As DFs are complex polysaccharides with differing structures and conformations it is not unsurprising that different DFs have different hydration characteristics. Conventionally these have been investigated in terms of water absorption by filtration (water holding capacity) or by centrifugation of hydrated molecules (water binding capacity/free or available water). The understanding of these characteristics are crucial in developing efficient food processes

Table 2.3. Hydration characteristics of some Dietary Fibres (Guillon & Champ, 2000; Robertson *et al.*, 2000).

Source of fibre	Treatment	Particle size (μm)	Swelling (mlg^{-1})	Water retention	Water absorption
				(g water g^{-1} dry pellet)	(ml water g^{-1} dry DF)
Wheat bran	-	900	11.9	6.8	1.0
	-	320	5.9	3.0	0.9
	Native	-	7.0	7.0	
	Delignified	-	11.0	10.4	
	Extruded	-	9.0	4.4	
Oat bran	-	-	5.5	3.5	
Resistant starch					
Novelose	-	40	5.6	2.9	3.0

which may deliver consumer acceptable food products. For instance DFs with high water holding capacity can be used not only as DF enrichment, but also as functional ingredients to reduce calorific value, avoid syneresis and modify the viscosity and texture of the final product (Holtekjølén *et al.*, 2008; Goldstein *et al.*, 2010).

The water absorption of a DF gives an indication of substrate pore volume, and could help in understanding DF performance as it transits the gastrointestinal tract (Guillon & Champ, 2000). The faecal bulking capacity of DFs is also thought to be related to their water absorption/retention characteristics as well as their impact on microbial proliferation (Davidson & McDonald, 1998). Yet this is contradicted by certain DF's such as pectin which has a high water capacity compared with wheat bran, this latter however has a stronger influence on faecal bulking as it retains its structure due to poor fermentability (Slavin, 2010).

2.7.5 Solubility of dietary fibres

The functional and nutritional properties of DF are intrinsically linked to their solubility characteristics and hence molecular conformation. The most common form of molecular shape of DF is as some sort of helical shape (BeMiller & Whisler, 1996). It is not unknown for certain DFs to possess chains which adopt regular, ordered conformations and pack together into crystalline-solid assemblies; this type of polymer is likely to be more stable in the solid state than in solution (Guillon & Champ, 2000). As the chemical regularity of a chain increases so too does the strength of the links, conferring insolubility, and hence resistance to enzymatic attack. This is the reason why linear structures such as cellulose

(generally viewed as having a flat ribbon-like conformation) undergoes limited digestion during enzymic exposure. Conversely, a more highly branched or irregular structure confers weaker links and tends to be more susceptible to degradation. Polysaccharides with greater branching hydrate more easily and are therefore regarded as soluble.

The ionic charge on a polysaccharide branch chain will affect solubility. Neutral polysaccharides, such as cellulose and starch, have a strong tendency for self-association. Pectins, however, contain charged groups within the molecule consequently promoting solubility due to the electrostatic repulsion, which inhibits the formation of ordered arrangements. Solubility is also affected by temperature. A temperature increase will cause the structure of a formerly insoluble polysaccharide to open up with a consequently loss of stability hence allowing it to solubilise as it becomes less well ordered.

The physiological effects of a DF seem to be derived from its solubility. For instance soluble viscous DFs have been associated with carbohydrate and lipid, while insoluble DF generally contributes mainly to faecal bulk improving bowel habits (Jenkins *et al.*, 2001; Kritchevsky, 2001). The determination of solubility has been important to enable classification into water-soluble (SDF) and water insoluble DFs (IDF).

The food industry often uses soluble DFs to modify the properties of liquid food products (textural properties such as mouth feel and stability). Due to the fact that these soluble components generally form viscous matrices, they are commonly referred to as gums or hydrocolloids. Their use is normally at relatively low concentrations (0.25-0.5 %) as higher levels can cause too much stickiness of a product (Brennan, 2005). This viscosity development property of SDF is important in both the manipulation of food structure and texture and also how they contribute to overall nutrient availability and digestion. Viscous solutions can change the rheology of the intestinal contents (Knudsen & Lærke, 2010)

Table 2.4 illustrates the range of effects SDF and IDF have on the glycaemic impact of foods (blood glucose levels). Evidence from the collection of peer-reviewed publications indicates that the addition of certain fibre components may have a beneficial effect both on an *in vitro* and an *in vivo* basis. Most research illustrates a reduction in glucose levels associated with fibres such as guar gum, psyllium and β -glucan (those fibres which are known to affect the viscosity profile of foods). It is possible therefore that the association between viscosity altering behaviour of the gums and the effect on starch digestibility are associated. This point will be discussed in more depth later on in this thesis.

Table 2.4. The effect of DFs on glycaemic and insulinaemic responses - studies *in vivo* and *in vitro*

Dietary fibre/ level used	Studies <i>in vivo</i>		Studies <i>in vitro</i>		Reference
	Glucose/insulin levels	GI	Glucose/ reducing sugars	GI/ HI	
Guar gum (2.5, 7.5, 12.5g)	↓* insulin	-	-	-	(Torsdottir et al., 1989)
Guar gum 20g/100kcal vs 4g/100kcal (on healthy subjects)	↓ glucose ↓ plasma glucose	↓ GI (with aprox. - 30%)	-	-	(Benini et al., 1995)
Guar gum (molecular weights and particle sizes) healthy volunteers	no effect on plasma glucose level ↓ plasma insulin	-	-	-	(Ellis et al., 1991)
Guar gum at 20 or 40 g/kg (on pigs)	↓ blood glucose	-	-	-	(Ellis et al., 1995)
Guar gum 3.4% on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
Guar gum, xanthan gum, methylcellulose, wheat bran - 70g (on rats)	↓ blood glucose for meals - containing viscous DF	-	-	-	(Cameron-Smith et al., 1994)
Guar gum <i>In vitro</i> and <i>in vivo</i> (on pigs)	↓ blood glucose	-	↓ glucose produced	-	(Brennan et al., 1996)
Guar gum, xanthan gum, CMC, water - insoluble DF, water soluble DF, resistant		-	↓ glucose	-	(Ou et al., 2001)
Guar gum (molecular weights) 7.6g <i>per</i> serving - NIDDM patients	↓ blood glucose ↓ blood insulin	-	-	-	(Gatenby et al., 1996)
Guar gum (60g TDF of which 15g were guar gum vs 16g TDF)	↓ blood glucose level	↓ GI	-	-	(Lafrance et al., 1998)
Guar gum 6.3g <i>per</i> serving	↓ blood glucose ↓ blood insulin	-	-	-	(Fairchild et al., 1996)
Inulin 10 g fed half way through the meal	No effect ↓ blood glucose	-	-	-	(Rumessen et al., 1990)

Inulin (15g/day) (patients with type 2 diabetes)	No effect	-	-	-	(Alles et al., 1999)
Inulin 8g/day	↓ fasting blood glucose	-	-	-	(Yamashita et al., 1984)
Inulin 10g/day (on healthy volunteers)	↓ fasting insulin level	-	-	-	(Jackson et al., 1999)
Pectin Liquid diet 2.5%	↓ maltose absorption	-	-	-	(Chun et al., 1989)
Pectin 3.4% on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
Cellulose 10%	↓ blood sugars (-5%)	-	-	-	(Mahapatra et al., 1988)
Cellulose 3.4% on dmb (on dogs)	no effect on plasma glucose	-	-	-	(Diez et al., 1998)
I. Psyllium and mixture psyllium-citrus pectin (2.2g)	I. no effect	-	-	-	(Frape and Jones, 1995)
II. Sugar beet fibre (6g) and cellulose (2g)	II. ↓ blood glucose level ↓ blood insulin level				
Sugar beet fibre (on healthy subjects)	↓ blood glucose level	-	-	-	(Thorsdottir et al., 1998)
Psyllium 2.5%	reduced fasting glucose levels	-	-	-	(Watters and Blaisdell, 1989)
Psyllium 7.4 g (healthy volunteers)	↓ insulin level ↓ blood glucose level	-	-	-	(Rigaud et al., 1998)
Psyllium 15g (healthy volunteers)	↓ blood glucose level	-	-	-	(Cherbut et al., 1994)
Wheat bran 15g (healthy volunteers)	no effect	-	-	-	(Cherbut et al., 1994)
Wheat bran 12g/day	flatten glucose	-	-	-	(Kritchevsky, 1988)

Wheat bran (6%) In pigs	No significant effect on blood - glucose or insulin levels		Increased the - rate of hydrolysis	(Leclere <i>et al.</i> , 1993)
Beet fibre (6%) In pigs	No significant effect on blood - glucose or insulin levels		Decreased <i>in vitro</i> hydrolysis	(Leclere <i>et al.</i> , 1993)
Sugar beet fibre 15g (healthy volunteers)	↓ blood glucose level	-	-	(Cherbut <i>et al.</i> , 1994)
Pea fibre	Flatten glucose	-	-	(Hamberg <i>et al.</i> , 1989a; Hamberg <i>et al.</i> , 1989b)
β-glucan from oats (4, 6 and 8g) (NIDDM subjects)	↓ blood glucose level ↓ blood insulin level estimate of 50% decrease in GI	-	-	(Tappy <i>et al.</i> , 1996)
β-glucan from barley (healthy subjects)	↓ blood glucose level ↓ blood insulin level	-	-	(Yokoyama <i>et al.</i> , 1997)
β-glucan from barley 15.7g TDF	No effect on glucose ↓ blood insulin level	-	-	(Bourdon <i>et al.</i> , 1999)
β-glucan from barley foods and cellulose from wheat foods 21-38 gTDF/day	No effect on glucose level	-	-	(McIntosh <i>et al.</i> , 1991)
β-glucan from barley <i>in vivo</i> (10 healthy volunteers) and <i>in vitro</i>	↓ blood glucose level ↓ blood insulin level	↓ GI	↓ reducing sugars ↓ HI released	(Granfeldt <i>et al.</i> , 1994)
β-glucan from oats (9 healthy volunteers)	No effect on glucose or insulin levels	no effect on GI	-	(Granfeldt <i>et al.</i> , 1995)
Oat bran - β-glucan <i>in vivo</i> and <i>in vitro</i>	↓ blood glucose level ↓ blood insulin level		↓ sugars released	(Holm and Bjorck, 1992)
Oat bran - β-glucan			↓ sugars	(Hudson <i>et al.</i> , 1992)
β-glucan : oat and barley products	↓ blood glucose ↓ blood insulin levels	-	-	(Liljeberg <i>et al.</i> , 1996)
β-glucan from oats (review paper)	↓ blood glucose level (50% - reduction for 10% β-glucan)	-	-	(Wursch and PiSunyer, 1997)

2.7.6 The glycaemic index and associated ways of determining the glycaemic response of meals

The concept of the glycaemic index (GI) has become a worldwide standard (Burton *et al.*, 2011) and is based on the postprandial increase in the plasma glucose concentration (ie, the glycaemic response) from a fixed amount of available carbohydrate in a test food as a percentage of the glycaemic response elicited from the same amount of carbohydrate in a reference food.

Glycaemic index is determined using selected subjects with standard BMI and physiological responses who have fasted overnight. In these normal healthy subjects a capillary finger prick sample is taken when fasting and then at 15, 30, 45, 60, 90 and 120 min after the start of the test meal (Wolever *et al.*, 1991a). The reference food should ideally be 50 g of glucose due to the rapid utilisation of glucose in the body (Burton *et al.*, 2011). However white bread containing 50 g available carbohydrate is often used as it is considered to be more physiologically similar to food during digestion and is less likely to cause nausea after consumption compared to a glucose drink. The reference and test foods are fed to subjects on separate occasions in a random order and the area under the glycaemic response curves (AUC) is determined (Wolever *et al.*, 1991a). GI is calculated as $GI = (AUC_{\text{food}} / AUC_{\text{glucose}}) \times (Wt \text{ glucose} / Wt \text{ available carbohydrate in food}) \times 100/1$ (Monro & Shaw, 2008). Where white bread is used as the reference food, the GI_{wb} must be converted by dividing GI_{wb} by 100/x (where x is the mean GI value of white bread) (Wolever, 2006).

Although GI has gained consumer acceptance as a nutritional measure it is actually unitless. This fact (that it has no units) means that it is unrelated to portion size so bears no resemblance to the calorific value of a food. Additionally, the GI value of a food may not be consistent, for example although the white bread is standardised for 50 g available carbohydrate, the procedure does not account for batch to batch variation in bread samples which may occur due to difference in flour quality (starch and protein content) as well as mechanical mixing. Difference in flour quality and mixing parameters result in dynamic variations in water absorption capacity of doughs leading to differing moisture levels of the bread, this in turn may contribute to variations in the structure and texture of the bread, as well as starch gelatinisation characteristics. Thus batch to batch variation and bread storage conditions, can result in different glycaemic responses (Venn & Green, 2007; Burton *et al.*, 2011). Essentially the GI value is a measure of response to 50 g of available carbohydrate.

Available carbohydrate in this context is measured using a finely ground food sample. More recent research has demonstrated that finely grinding of food samples destroys the natural integrity of the food structure in a different mechanism to mastication and thus can lead to overestimation of carbohydrate digestibility (Woolnough *et al.*, 2010; Burton *et al.*, 2011).

In spite of attempts to standardise procedures in the determination of GI, there are well documented cases of person to person, within person and ethnic (Wolever *et al.*, 1991a; 2002) variability affecting results as well as sample sizes for the purposes of GI determination of food products being relatively small n=10 is the minimum recommended sample (Brouns *et al.*, 2009; GI Symbol website 2012) size however Foster-Powell *et al.*, (2002) report that frequently the sample size is n=8 and may be as low as n=5.

While subjects are required to fast overnight before consuming a test meal there is evidence that the meal consumed the previous evening can affect glycaemic response the following day (Wolever *et al.*, 1988; Nilsson *et al.*, 2006)

Research has also indicated that where sample sizes of less or greater than 50 g are used and then converted to values equivalent to 50 g, the process of conversion of glycaemic response may lead to errors occurring. This is due to the fact that the glycaemic response of individuals to a 25 g portion of glucose (available carbohydrate) is not linearly proportional to the response to 50 g and leads to a rapid undershoot of values (values return to below baseline sooner for 25 g samples compared to 50 g samples) (Brand-Miller *et al.*, 2009). Similarly, researchers have also indicated that glucose loadings over 50 g are not linearly correlated to a 50 g response leading to a potential overestimation of GI (Wallace *et al.*, 2006). These points indicate that the determination of GI may not be correlated to the glycaemic load (GL) of a food product.

2.7.7 Carbohydrate digestion and *in vitro* simulations

Traditionally the factors surrounding the assimilation of carbohydrate foods were considered to be relatively uncomplicated. Such foods were considered to be either simple (sugars) or complex (starches) carbohydrates. Sugars (mono- and di- saccharides) are most easily absorbed causing large and rapid rises in blood glucose after ingestion. Complex carbohydrates are digested more slowly and produce flat blood glucose responses. However it has become apparent that there are many variances as to how the different

classifications of carbohydrate exert their effects on the digestive system (Jenkins *et al.*, 1986).

Digestion of carbohydrates commences in the mouth with the teeth mechanically breaking down the foods as they are mixed with saliva which contains α -amylase. The resulting bolus is suitably lubricated for swallowing and allowing limited hydrolysis of starch to maltose and dextrins (Simpkins & Williams, 1992).

Swallowed boluses are propelled via peristaltic contractions through the oesophagus and into the stomach where gradual mixing with acidic (\sim pH 2) gastric secretions occurs. Pepsin enzyme, secreted by the stomach digests the protein component of the meal. The acidic conditions of the stomach inactivate salivary α -amylase preventing further hydrolysis of starch, although it is likely that a small amount of starch digestion continues within the centre of undisrupted food boluses provided α -amylase is protected from gastric conditions (Sherwood, 1997). Rhythmic contractions of the stomach wall reduce solid bolus matter into a creamy acid suspension called chyme (Simpkins & Williams, 1992).

Gastric emptying into the duodenum occurs in periodic spurts of chyme that are rapidly mixed (via duodenal contractions) with proteolytic enzymes, lipases, pancreatic α -amylase, sodium bicarbonate and bile salts (Sherwood, 1997). Once in the small intestine, pancreatic α -amylase enzyme hydrolyses the α -1,4 glycosidic bonds of starch producing glucose, maltose and dextrin (monosaccharide, disaccharide and oligosaccharides). Disaccharides are hydrolysed, into their more readily absorbed monosaccharide components, by disaccharidase enzymes which are secreted by the small intestine wall. As starch is the major carbohydrate in the human diet, up to 80 % of monosaccharides absorbed within the small intestine, are glucose (Caspary, 1992; Gray, 1992). **Figure 2.7** provides a summary of the major processes underlying carbohydrate digestion in the human upper gastrointestinal tract and generation of a glycaemic response.

In the last two decades a variety of *in vitro* methods to analyse digestibility of carbohydrates within food have been described. The predominant theme of these *in vitro* procedures is that they aim to imitate physiological conditions however the way in which this is done varies considerably.

In vivo digestion is initiated by chewing. Chewing can be a very individual characteristic and involves disrupting the food structure while incorporating saliva to allow an amount of starch hydrolysis by α -amylase. Chewing has been mimicked in many ways, procedures

have engaged the use of sieves with varying gauges (Karkalas, 1985; Brighenti, *et al.* 1998), mincers (Englyst *et al.*, 1999; Araya *et al.*, 2002) or food processors (Brennan *et al.*, 1996). Analyses not necessarily requiring realistic “as eaten” food particle sizes, sample foods may be ground, milled or homogenised (Champ, 1992; Goñi, 1997; Weurding *et al.*, 2001).

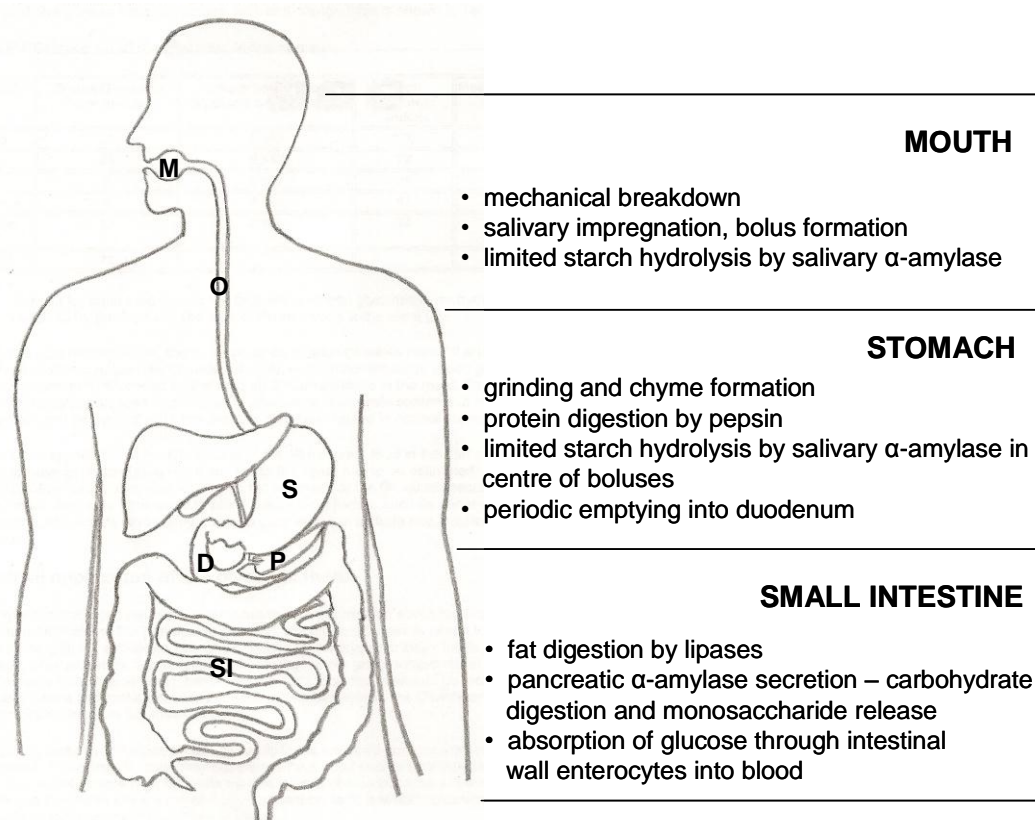


Figure 2.7. The processes underlying carbohydrate digestion and generation of a glycaemic response within the human upper gastro-intestinal tract. M, mouth; O, oesophagus, S, stomach; D, duodenum; P, pancreas; SI, small intestine. (Woolnough, 2011).

Protein digestion is an intrinsic part of the digestion process that occurs within the acidic churning of the stomach itself. Gastric emptying of chyme into the duodenum is affected by variables such as food quantity and viscosity (Turnbull *et al.*, 2005; Horner *et al.*, 2011)

In vitro procedures also began to include a pepsin proteolysis step. This is important in relation to total and resistant starch assays, as complete starch digestion can only be achieved through the disruption of the protein – starch matrix. (Holm *et al.*, 1986; Granfeldt *et al.*, 1992). On entering the duodenum, chyme is mixed with pancreatic secretions which contain the efficacious enzyme α -amylase. The starch is hydrolysed while

the small intestine continues to mix the chyme and secretions with strong contractions as well as propelling it along via peristalsis. The brush-border of the small intestine secretes the (maltase) enzymes required to complete the hydrolysis of the starch fragments into glucose. The glucose is absorbed across the intestinal wall and into the portal blood from where it is circulated into the entire body contributing to the glycaemic response.

2.7.8 Glycaemic Index in relation to dietary fibre content

As mentioned previously (**section 2.7.6**), the concept of GI is based on the postprandial increase in the blood glucose concentration (ie, the glycaemic response) from a fixed amount of available carbohydrate in a test food with the glycaemic response elicited from the same amount of carbohydrate in a standardised reference food. Foods with high levels of carbohydrate (derived from wheat flour) tend to be regarded as high GI products as a consequence of ease with which starch is digested. Diets consisting largely of high GI foods have been linked to the health damaging artefacts of poor insulin and blood glucose control, increased weight gain and increasing obesity (Jenkins *et al.*, 1997; Brennan, 2005).

Jenkins *et al.*, (1987) showed that diets rich in SDF, such as guar gum, pectin and sugar beet fibres, result in lowered postprandial blood glucose and insulin levels. Insoluble NSP has a limited effect on GI (Jenkins *et al.*, 1997). Soluble NSP, present in pulses, vegetables, whole fruits, oats and barley, are able to form thick gels inside the stomach thereby delaying gastric emptying and so to enzymic digestion via the physical barrier formed around the carbohydrate (Jenkins *et al.*, 1978; Chaplin, 2003). Gastric emptying rate appears to be little affected by IDF consequently there is no effect on glucose absorption. Therefore it is apparent that a high DF diet may not be synonymous with low glycaemic index foods (Jenkins *et al.*, 1983).

The most frequently occurring DF in food products, including breakfast cereals, wholemeal bread and brown rice, is cellulose. Due to the insoluble nature of cellulose the GI remains similar despite the increase in DF (Jenkins *et al.*, 1983). There does however appear to be an exception to this observation, Kellogg's All-Bran, an extruded wheat bran product. "All-Bran" has a high IDF with a low GI, the mechanism for this is not yet understood but could be due to the complexing of DF and carbohydrate altering starch digestibility. It is possible, however, to influence the digestibility of starch in breakfast

cereal products by altering the soluble DF composition of extruded breakfast cereals (Brennan *et al.*, 2008a)

Wolever and Jenkins (1986) also discovered that dietary carbohydrates can impact on the glycaemic response of a later meal, finding a reduced glycaemic response to a lunch time meal when it is preceded by a low GI compared to a high GI breakfast. Similarly Björck and Elmståhl (2003) reported a glycaemic impact of DF from one meal to another, where an evening meal rich in NSP, with a low glycaemic index will result in an improved glucose tolerance the following morning. It is therefore possible that the consumption of a low GI breakfast could have advantages in the regulating the glycaemic response of meals throughout the day. One of the major problems with this suggestion is that many extruded snacks tend to be regarded as high GI food products (Foster-Powell, 2002). This is in part due to cereals being the major component of extruded snacks and hence the snacks having a high carbohydrate content, it is also in part related to the extrusion process which alters the chemical composition of the food product, and the digestibility of the starch within the carbohydrate food products.

The challenge for the food industry therefore is to develop high DF cereal based extruded snack products which show low postprandial glucose response. In achieving this the food industry needs to understand the relationship between DF ingredient characteristics and their potential utilisation in food processing. This represents the focus of this thesis as detailed in the aims of the project (**section 1.3**):-

1. Evaluate suitable commercially available DF and DF in the co-products of food waste stream production for inclusion into fibre rich extruded snack products.
2. Analyse and determine the relationship between the incorporation of fibre-rich materials into extruded food products and their effects on the physicochemical properties of the food products and sensory analysis characteristics.
3. Evaluate the effect of extrusion technology on the nutritional composition of the extruded snack products (namely starch digestibility and glucose release).
4. Compare and contrast the glycaemic response of fibre rich products recorded using *in vivo* and *in vitro* analysis.

Successful completion of the research will be a significant benefit to the food industry.

Chapter 3

Materials and Methods, Phase One.

3.1 Introduction.

This chapter describes the basic experimental methodology used in the evaluation of the extruded products (and their raw mixes) used throughout this thesis. Essentially the research was divided into two main experiments. The first experiment was to investigate 8 potential fibre, and fibre-rich, components as materials to be used in the production of fibre-rich extruded snack products. Both chemical and nutritional analysis were performed on the 23 sample products developed after initial desktop screening of potential sources. The second main experiment was to develop two concept products containing a high inclusion rate of fibre and to assess these products against a control recipe in terms of their chemical and nutritional characteristics. Throughout the experiments sensory analysis using untrained panellists was used on selected products (as explained in the thesis) so as to evaluate the consumer acceptability of the snack products manufactured against a control product. In addition, the final experiment was designed to use the selected samples in an *in vivo* (pseudo-clinical) trial to evaluate the potential blood glucose regulating effects of fibre enriched extruded snack products.

3.2 Food grade materials used in the manufacture of extruded products.

Extruded snack products were made using a base recipe incorporating wheat flour, maize grits and oat meal (**Table 3.1**). The base recipe had been obtained from a New Zealand breakfast cereal manufacturer in 2007 (supplier requested to be kept confidential). Previous research by myself had used this recipe for my M. Phil. research conducted at Massey University, Palmerston North, New Zealand and presented in my M. Phil. thesis (Brennan, 2008) as well as my previous research publications on the interactions of novel dietary fibres and extruded foods (Brennan *et al.*, 2008 a,b).

For this study, white culinary wheat flour and was obtained from Smith's Flour Mills (Worksop, Notts, U.K.). Oat bran and barley flour were obtained from Little Salkeld Mill (Cumbria, U.K.) and fine oat meal was obtained from Morning Foods Ltd (Crewe, U.K.).

Fine maize grits were supplied by Dacsa, Seaforth Corn Mill (Liverpool, U.K.). These flours were used as the major constituent of the base recipe.

Non-starch polysaccharide was used in the base recipe at 15 %, 10 % and 5 % as a replacement of the wheat flour. The NSPs used were psyllium husk, inulin, β -glucan derived from barley, oat bran, concentrated mushroom waste material, super gum (gum acacia material) and gum gatifolia. These materials were selected as NSPs which had been reported in literature as having potential uses in the food industry (psyllium and inulin as texturising agents) or possessing potential functional nutrients (barley and oat products related to cholesterol lowering effects, super gum and gum gatafolia related to potential renal modulating effects, and mushroom material for a potential immunological role).

Psyllium husk was purchased from Holland and Barrett (Nuneaton, U.K.). Inulin was obtained from Orafit Active Food Ingredients (Oreya, France). Super Gum EM 10 and Gatifolia SD were supplied by San-Ei Gen F.F.I. (Tokyo, Japan). Concentrated Barley Beta-Glucan was supplied by Polycell Technologies (Hamburg, Germany). Mushroom waste was supplied by Oakfield Farm Products (Evesham, U.K.) and material extracted as described, in **section 3.3**, below. Additionally an extruded snack was produced with total replacement of wheat flour with barley flour to determine the potential of using barley flour as the main ingredient in extruded snacks instead of wheat flour, giving a product with 65 % barley flour.

Table 3.1. Recipes used to determine best fibre/waste to use in extruded product.

Substitution level	Wheat flour (g/100g)	Maize grits (g/100g)	Oatmeal (g/100g)	Experimental ingredient (g/100g)
Control	65	20	15	0
5%	60	20	15	5
10%	55	20	15	10
15%	50	20	15	15
65%	0	20	15	65

3.3 Extraction and preparation of waste stream material from spent mushroom compost.

Stalks of chestnut mushrooms (**Figure 3.1**) were obtained from Oakland Farms (Evesham, U.K.). The stalks were the waste residue after harvesting of the mushroom cap had occurred.

The stalks were collected, separated (**Figure 3.2**) and cleaned of any compost/growing medium by hand removal of clumps of soil, followed by careful brushing of the stalks. The cleaned stalks were then freeze dried for 2 days using an Edwards Super Modulyo freeze drier (Bristol, U.K.). The freeze dried stalks were hermetically sealed and stored at ambient temperature.



Figure 3.1. Image of mushroom waste as recovered from Oaklands farms.



Figure 3.2. Image of mushroom stalks selected for cleaning and recovery of fibre components.

Cleaned, dried stalks were milled to produce a powder using a Retsch ZM100 with a number 2 screen (Retsch GmbH, Haan, Germany). The resulting powdered mushroom waste material was hermetically sealed in foil bags and stored at room temperature for future use.

3.4 Extrusion processing used for preliminary evaluation of the effect of differing levels of eight dietary fibres into extruded snack products.

A Werner Pfleiderer (Stuttgart, Germany), Continua 37, co-rotating, self wiping, twin screw extruder was used to manufacture the product (**Figure 3.3**).



Figure 3.3.Werner Plfeiderer, Extruder, Continua 37.

The L/D (length to diameter) ratio was 27:1, the screw diameter was 37.4 mm, using Werner and Pflfeiderer screw profile number 2054 (maximum torque 90 Nm) and a screw speed of 175 rpm was maintained throughout the extrusion process.

Extrusion was conducted at 9 kg/hr and the feed rate of the samples was determined by calibrating each sample through the feed hopper (Rospen Twin Screw Volumetric Feeder, Gloucester, U.K.) as a dry mix prior to extrusion and recording the actual mass passing through the hopper as a factor of time.

A 4 mm diameter twin die was used for all samples. Temperature and pressure recorders were positioned at the die face to determine exit temperature and pressure. **Table 11.1** (appendix) shows the pressure, torque and temperature recorded during the extrusion of all samples.

An automated product cutter was placed at the die face and set at 300 rpm in order to obtain pelleted snack product cereal. Expanded snack products (collets) were allowed to air dry and cool for 1 hr before sealing in plastic bags for storage.

Chapter 4

Determination of Physicochemical Properties of Phase One Unprocessed (raw) Mixes and Extruded Products.

4.1 Moisture content of raw and extruded samples

Moisture determinations of raw product base and extruded products were conducted according to the AACC methodology (Moisture-Air-Oven Methods, Method 44-15A) (AACC 2000) based on oven drying for 1 hr at 130 °C or at 70 °C until constant weight was achieved. Moisture was recorded for the raw mixes as well as the extruded samples (**Table 11.3** in appendix). The moisture loss during extrusion was calculated as the difference between original moisture level of the raw sample and the moisture content of the extruded sample expressed as a percentage of the original moisture content.

4.2 Total starch determination.

Total starch was determined in duplicate using the Total Starch assay kit from Megazyme International (Wicklow, Ireland) (McCleary *et al.*, 1997). Each sample (100 mg) was suspended in 5 mL ethanol (80 %) and incubated at 80 °C for 5 min, the resulting suspensions were mixed on a vortex and further 5 mL ethanol (80 %) were added to each tube. Each tube was then centrifuged (using a Sanyo MSE Centaur 2 centrifuge) for 10 min at 1000 g. The supernatant was discarded and the pellet resuspended in 10 mL ethanol. This was centrifuged again at 1000 g and again the supernatant was discarded after which 3 mL MOPS buffer/thermostable α -amylase solution was added. The sample was mixed with a vortex mixer and boiled for 6 min (stirred after 2 and 4 min). The sample was then placed in a 50 °C water bath and 4 mL sodium acetate added followed by amyloglucosidase (0.05 mL, 20 U), mixed using a vortex and put back into the 50 °C water bath for 30 min. The contents were then transferred to a 100 mL volumetric flask and made to volume with distilled water. The flask was mixed well and an aliquot taken. The aliquot was spun at 1000 g for 10 min. Duplicate aliquots (0.1 mL) of the diluted solution were transferred to the bottom of a glass test tube to which 3 mL of Glucose oxidase/peroxidase mixture (GOPOD) was added and the tube incubated at 50 °C for 20

min. Control samples were run with glucose standard solution and a blank with water. Absorbance was measured at 510 nm.

4.3 β -Glucan assay

Two different β -glucan assays were carried out due to β -glucan having a different structure in mushroom to that in cereals.

4.3.1 β -glucan assay, mixed linkage

β -glucan was determined using 'Mixed-linkage Beta-glucan' assay procedure, stream lined method, from Megazyme International (Wicklow, Ireland). The method is adopted by the American Association of Cereal Chemists (AACC Method-32-23) as the official method for the determination of mixed linkage [(1-3)(1-4)]- β -D-glucan in oat and barley flour and fibre samples (McCleary & Codd, 1991). A sample of 0.2 g was placed into a 15 mL centrifuge tube, to which 5 mL, 50 % ethanol was added and the tube incubated in a boiling water bath for 10 min. The tube was mixed on a vortex and a further 5 mL, 50 % ethanol was added before being mixed on the vortex again. The tube was centrifuged at 1000 g for 10 min and the supernatant discarded. Four (4) mL, 20 mM, pH 6.5 sodium phosphate buffer was added to the tube then it was mixed on a vortex. The tube was heated in a boiling water bath vortexing after 2 min and 4 min. The tube was then incubated at 50 °C and allowed to equilibrate for 5 min. Lichenase (0.2 mL) was then added and mixed before incubating at 50 °C for 60 min and stirring vigorously 3-4 times during that time, after which 2 mL, 200 mM, pH 4 sodium acetate buffer was added and mixed on the vortex. The tube was allowed to equilibrate for 5 min at room temperature before centrifuging for 10 min at 1000 g. Aliquots of 0.1 mL of the supernatant were dispensed into the bottom of three test tubes. To two of the tubes 0.1 mL beta-glucosidase was added and to the third 0.1 mL, 50 mM acetate buffer was added. All three tubes were incubated at 50 °C for 10 min, then 3 mL GOPOD Reagent was added to each tube and they were incubated at 50 °C for 20 min. The tubes were removed from the water bath and the absorbance measured at 510 nm within 1 hr.

4.3.2 β -Glucan, mushroom and yeast

Mushroom and yeast β -glucan is structurally different to cereal β -glucan so a different assay procedure was required to assay the β -glucans in the mushroom waste samples. The Mushroom and Yeast Beta-Glucan assay from Megazyme International (Wicklow, Ireland) was used. The β -glucan in the mushroom waste samples would have different linkages namely, [(1-3)(1-6)]- β -glucan, they did not show up in the previous assay which only identified[(1-3)(1-4)]- β -D-glucan.

Firstly total glucan was determined. A 100 mg milled sample was weighed into the bottom of a glass culture tube. Concentrated hydrochloric acid (37 % v/v) (1.5 mL) was added to the tube, the cap was fitted to the tube and the tube was vigorously mixed on a vortex mixer. The tube was placed in a water bath at 30 °C for 45 min with mixing every 15 min on a vortex mixer. Water (10 mL) was added to the tube and it was mixed on a vortex. The cap was loosened and the tube was placed in a boiling water bath, after 5 min the cap was tightened and the incubation continued for 2 hr. The tube was cooled to room temperature and 10 mL 2 N KOH was added. The contents of the tube were quantitatively transferred to a 100 mL volumetric flask using 200 mM sodium acetate buffer (pH 5.0), made to volume and mixed well. An aliquot was centrifuged at 1500 g for 10 min using a Rotana 460 R centrifuge. From the resulting supernatant an aliquot of 0.1 mL was transferred to each of two test tubes. A mixture of *exo*-1,3- β -glucanase (20 U/mL) plus β -glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 5.0) (0.1 mL) was added to each tube and they were mixed on a vortex before incubating at 40 °C for 60 min. GOPOD (3 mL) was added to each tube and incubated at 40 °C for 20 min. The absorbance of the solutions was measured against a reagent blank at 510 nm. All samples were analysed in duplicate and against a reagent blank.

Secondly α -glucan was determined, this was subsequently subtracted from the total glucan measurement to give a measure of β -glucan. A 100 mg milled sample was weighed into a glass culture tube. A magnetic stir bar was added and 2 mL of 2 M KOH, the sample was suspended by stirring for 20 min in an ice bath over a magnetic stirrer. While stirring 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added by followed 0.2 mL of amyloglucosidase (1630 U/mL) plus invertase (500 U/mL). The tube was mixed using a vortex and placed in a water bath at 40 °C for 30 min with intermittent mixing using a vortex mixer. The contents of the tube were quantitatively transferred to a 100 mL and made to volume with water, it was mixed well before an aliquot was centrifuged at 1500 g

for 10 min using a Rotana 460 R centrifuge. From the resulting supernatant an aliquot of 0.1 mL was transferred to each of two test tubes. To each tube 0.1 mL of 200mM sodium acetate buffer (pH 5.0) and 3 mL GOPOD were added. The tubes were incubated at 40 °C for 20 min. The absorbance of the solutions was measured against a reagent blank at 510 nm. All samples were analysed in duplicate and against a reagent blank.

The results from this second assay were not consistent, probably due to the presence of cellulose in the sample, help was sought from Megazyme, however due to lack of consistency the results are not presented.

4.4 Dietary fibre determination using Megazyme International analysis

Soluble and insoluble dietary fibre contents were determined using the Total Dietary Fibre assay procedure from Megazyme International (Wicklow, Ireland). The method is adopted by the American Association of Cereal Chemists (AACC Method-32-07) as the official method for the determination of soluble, insoluble, and total dietary fibre in foods and food products (Prosky *et al.*, 1992).

4.4.1 Insoluble dietary fibre determination

The sample was weighed in quadruplicate (this allowed for duplicate ash and protein determinations) into 400 mL beakers, 40 mL MES-TRIS pH8.2 buffer was added to each beaker and stirred on a magnetic stirrer until there were no lumps. While stirring at a slow speed 50 µL α -amylase was added to each beaker. The beakers were covered with aluminium foil then incubated for 35 min at 95-100 °C in a shaking water bath.

After cooling to 60 °C the foil covers were removed, the gel in the bottom and the rings on the side wall were scraped with a spatula and the side walls rinsed with 10 mL distilled water. Protease solution (100 µL) was added then the beakers were covered with foil and incubated for 30 min at 60 °C in a shaking water bath.

The beakers were placed on a stirrer and the foil removed before 5 mL 0.561 N HCl was added, the pH was checked to be between 4.1-4.8. While stirring continued 200 µL amyloglucosidase was added, the beakers were covered with foil and incubated for 30 min. at 60 °C in a shaking water bath.

Fritted crucibles containing acid washed, pre-ashed celite were tared. The bed of celite was evenly distributed using distilled water and suction was applied to draw the celite onto the fritted glass as an even mat. The slurry was then filtered through the crucible into a filtration flask and the residue was rinsed twice with 10 mL of distilled water at 70 °C. The filtrates were saved and transferred to a pre-tared 600 mL beaker, these were then used for soluble dietary fibre (SDF) determination. The residues in the crucibles were each washed with 10 mL of 95 % ethanol and then 10 ml of acetone. The crucibles were then dried overnight in the oven at 103 °C. The dried crucibles were cooled in a desiccator for approximately 1 hour before weighing. The residues were then either analysed for protein using the Kjeldahl method or were ashed at 565 °C.

4.4.2 Soluble dietary fibre determination.

The SDF filtrates, (from 4.4.1) were made up to 80 g using distilled water and 320 mL of 95 % ethanol preheated to 60 °C was added.

Precipitates were allowed to form for 60 min at room temperature. Fritted crucibles containing acid washed, pre-ashed celite were tared.

The bed of celite was evenly distributed using 78 % ethanol and suction was applied to draw the celite onto the fritted glass as an even mat. The precipitates were filtered through the crucible into filtration flasks and the residues were each rinsed twice with 15 mL of 78 % ethanol, twice with 15 mL of 95 % ethanol and finally twice with 15 mL acetone. The crucible was then dried overnight in the oven at 103 °C.

The dried crucibles were cooled in a desiccator for approximately 1 hour before weighing. The residues were then either analysed for protein using the Kjeldahl method (2.4.9) or were ashed at 565 °C (2.4.10).

At the same time two reagent blanks were run to allow one to be analysed for protein and the other for ash.

4.4.3 Protein determination using the Kjeldahl method.

The dietary fibre crucible contents were scraped into a digestion tube. Two Kjeldahl tablets (each containing 3.5g K₂ SO₄ and 0.0035g Se) and 15 mL concentrated sulphuric acid were added. The tube was heated in a digestion block to 420 °C until the solution

became clear and removed from the heat after a further 20 min. The tube was cooled, and approximately 70 mL hot distilled water were added, before it was placed in a distillation unit. A conical flask containing 25 mL of 4 % boric acid solution (containing indicator) was placed under the condenser outlet. 30 mL of 40 % NaOH was dispensed in to the digestion tube and distillation lasted for 4 min. The resulting ammonium borate solution was titrated with 0.1 M hydrochloric acid, to a mauve/grey end point.

4.4.4 Ash determination.

The dietary fibre crucible was placed in an oven at 565 °C for 5 hours, the crucible was reweighed and percentage ash calculated.

4.4.5 Total dietary fibre

The IDF and SDF calculations were carried out using Megacalc (Megazyme International, Wicklow). While TDF was determined by taking the sum of IDF and SDF for each sample.

4.5 Water absorption index (WAI) and water solubility index (WSI)

Approximately 1g sample was weighed into a tared centrifuge tube and was mixed with 10 mL distilled water. The resulting slurry was vortexed for 1 minute and allowed to stand for 30 min. then centrifuged at 2000 g for 30 min. The supernatant was decanted into a pre-weighed evaporating dish which was then evaporated to a constant weight at 65 °C overnight, the tube containing the pellet was re-weighed.

WAI was expressed as mL water retained per g of sample and

WSI was the mass of dry solids in the supernatant expressed as a percentage of the original mass of sample.

4.6 Oil absorption index (OAI)

Approximately 1g sample was weighed into a tared centrifuge tube and was mixed with 10 mL corn oil (Mazola, CPC, England). The resulting slurry was vortexed for 1 minute and allowed to stand for 30 min before being centrifuged at 2000 g for 30 min (Carcea Bencini 1986). The supernatant was decanted and the tube containing the pellet was re-weighed. Oil absorption index was expressed as mL oil retained per g of sample.

4.7 Texture analysis of extruded products

The texture (hardness and fracture force values) of the extruded products was measured with a texture analyser (TA-XT32, Stable Micro Systems, Surrey, UK). Determination of the fracture behaviour of the extruded products was conducted using an aluminium cylinder of 35 mm diameter (P/35; Stable Micro Systems, Surrey, UK). The machine's test speed was adjusted to 0.5 mm/sec and the probe height calibrated to 14 mm.

Individual samples were placed centrally on the texture analyser platform (**Figure 4.1**) and the sample was then axially compressed until the probe had travelled 13 mm.

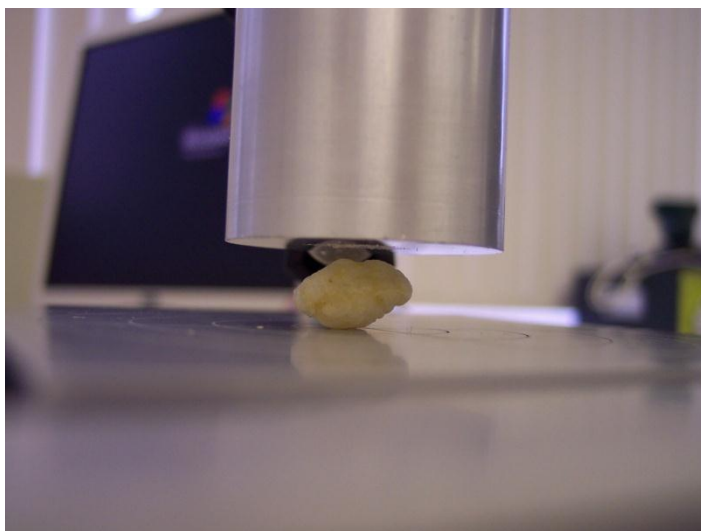


Figure 4.1. Illustration of sample underneath the probe of the texture analyser.

The trigger force for recording data was set at 5 g (determined by preliminary investigations to avoid false force recording). The peak mechanical force obtained during compression was recorded as the hardness of the cereal product. The number of peaks, greater than 50 g recorded during the compression, was recorded as the crispiness of the

product. The force 50 g was selected in consultation with Stable Microsystems, UK as the minimum level of force able to be applied without obtaining instrumental interference on the number of collected peaks.

The acoustic profile of the samples during extrusion was also determined using the machine. In order for the crunchiness / crispiness of the product to be evaluated an acoustic envelope detector (A/AED) was connected to the input of the machine and positioned 0.5 cm away from the extruded product during compression (**Figure 4.2**). The acoustic detector had the envelope corner frequency set to 3.125 KHz. The sound emitted during compression (in decibels (dB)) was recorded and the number of acoustic fracture events greater than 20 dB was obtained as the number of acoustic peaks. The value of 20 db was, advised by Stable Microsystems, to avoid picking up extraneous background noise. These were recorded as an illustration of the crispiness of the product.

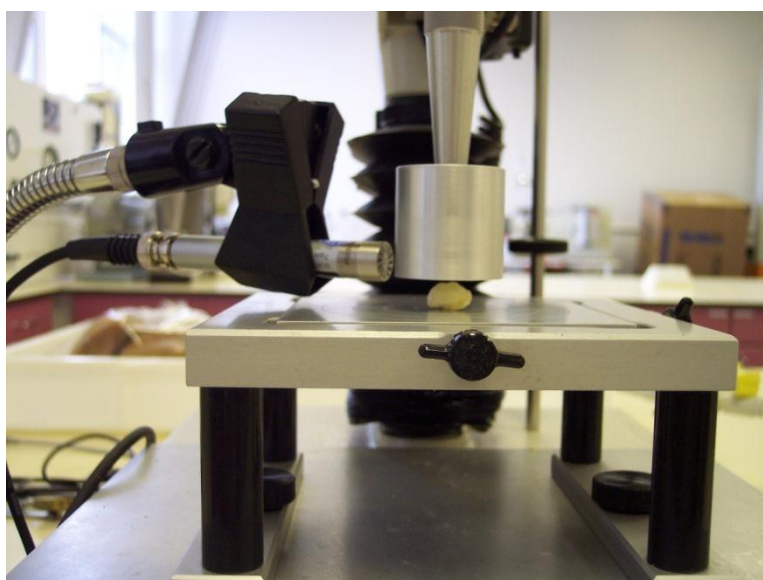


Figure 4.2. Sample on the texture analyser with acoustic envelope detector to the side.

During the compression run, the distance from point of contact with the collet (point at which the trigger force was detected) and the base plate of the texture analyser was recorded. This gave an accurate measurement of individual product heights. This information was used in the determination of product expansion (**section 4.8**).

All results were analysed using the software Texture Expert version 2,0,6,0 (Stable Micro Systems, Surrey, UK). All measurements were performed twenty times.

4.8 Percentage expansion of extruded products

Expansion of the product was expressed as a percentage of the diameter of the extruder die. The die diameter of the extruder was 4mm and the diameter of the extruded products was determined by the height of product recorded by the Stable Microsystems Texture Analyser (section 4.7). Values are expressed as a percentage of the original die diameter according to the formula below:

$$\text{Expansion Ratio} = \frac{\text{Average cereal collet diameter}}{\text{Die Diameter}} \times 100$$

4.9 Density determinations for extruded products

The weight of 1 L of extruded product was measured on a Mettler bench top balance to give the value of bulk density of the product. Product volume of the samples was determined by rape seed displacement method applied to 1 L of product. Simplistically the density is expressed as weight / volume :-

$$\text{Product density (kg/m}^3\text{)} = \frac{\text{Sample weight (g)} \times 1000(\text{ml/L}) \times 1000(\text{L/m}^3)}{\text{Sample volume (mL)} \times 1000(\text{g/kg})}$$

4.10 Determination of pasting properties of raw and extruded samples

The pasting properties of both the raw cereal bases and the extruded food products were determined using a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriwood, Australia). Samples were milled to achieve a fine particle according to the manufacturer's guidelines. Briefly, 25 mL of water was added to an aluminium canister for the RVA a sample suspension was prepared by adding 2.5 g of dry milled sample, the mixture was inverted and dispersed by shaking vigorously for 10 sec with a rubber stopper to avoid loss of product from the canister, the sample was then placed in the RVA apparatus with a plastic moulded paddle. Experiments were conducted at the paddle speed of 160 rpm and samples were subjected to the heating and cooling procedure of the standard 1 profile (see the example RVA profile in **Figure 4.3**) (Samaan *et al.*, 2006). The computer software Thermocline for Windows (Newport Scientific, Warriewood, Australia) was used to analyse the pasting profiles of graphs obtained. The peak viscosity (maximum viscosity of

the sample during the heating and holding phase of the procedure) and the final viscosity (viscosity readings at the end of the test profile) were recorded for all samples. Analysis of samples was conducted in duplicate.

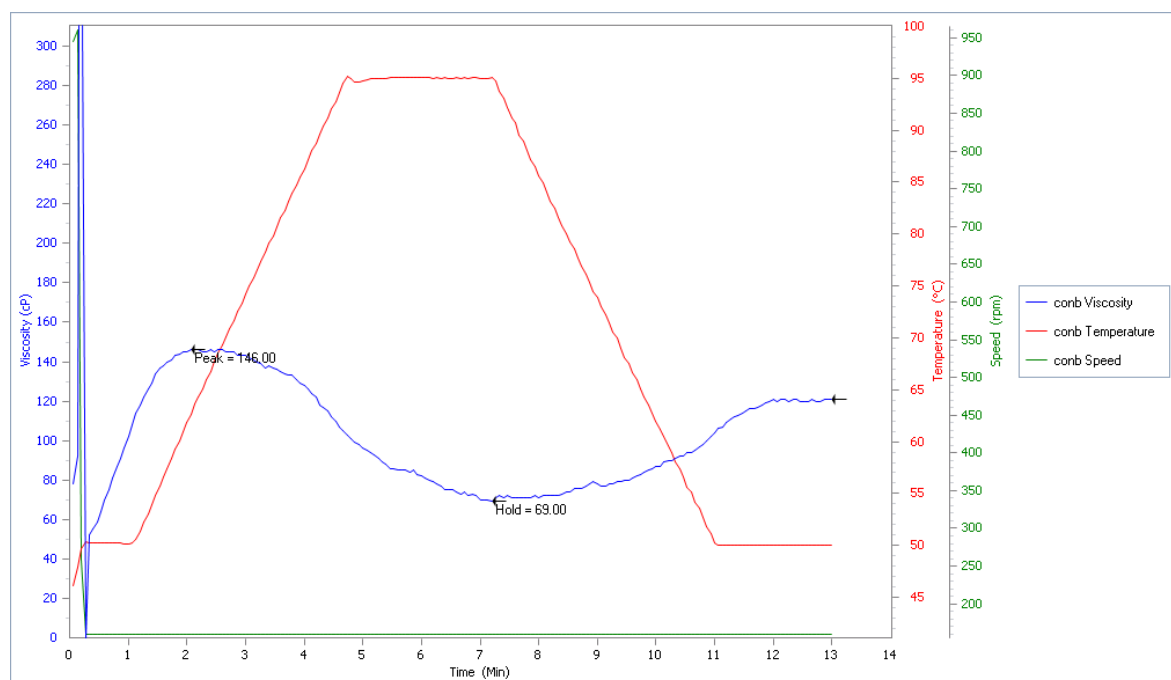


Figure 4.3. Example of a standard RVA profile

4.11 *In vitro* starch determination

In vitro starch hydrolysis was conducted in duplicate for each sample in the raw and extruded states in order to determine the potential amount of glucose released over a 120 min enzymatic protocol. The protocol used has been developed from a basic starch degradation procedure used by Brennan *et al.*, in 1996 through to a multi-enzyme simulation as described in detail by Woolnough *et al.*, (2010). The *in vitro* digestions simulated gastric and intestinal conditions and were carried out in 100 mL plastic biopsy pots placed on a pre-heated 15 place magnetic heated stirring block (IKA RT15) as shown in **Figure 4.4**.



Figure 4.4. *In vitro* digestion procedure.

Food samples (2.5 g), either raw base product or milled extruded food product were mixed with 30 mL of water and were held at 37 °C for 10 min. Pepsin (Fisher Scientific, P/1120/48 -1,000-1,500 u/ml) was then added (1 mL of 10 % solution in 0.05 M HCl). The containers were constantly stirred at 130 rpm and kept at 37 °C for 30 min to mimic gastric digestion.

After 30 min NaHCO_3 was added (2 mL of 1 M solution) and the pH adjusted to 6. At this stage a time zero 1 mL aliquot of the solution was taken to determine the amount of free available sugars in the product. The procedure continued with the addition of further enzymes. A 100 μL dose of amyloglucosidase EC 3.2.1.3. from *A.niger*, (Megazyme, E-AMGDF; 3260U/ml) (0.1 mL) was added to prevent end product inhibition of pancreatic α -amylase. Then 5 mL of 2.5 % pancreatin (Sigma, P1750) solution in 0.1 M maleate buffer (pH 6) was added to mimic ileal digestion. Maleate buffer 0.1M pH 6 was made by, dissolving 11.6 g maleic acid in 800 mL distilled water, this was adjusted to pH 6 using 4 M NaOH, 0.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added and 0.2 g sodium azide, the volume was adjusted to 1 L. The final digest volume was adjusted to 50 mL with distilled water. Aliquots of 1 mL were taken from the sample digests at time 0 (before adding enzymes to the substrate

samples), 20 min, 60 min and 120 min. Each 1 mL aliquot was mixed with 4 mL ethanol in a tube to stop the digestion and the tube centrifuged before reducing sugar content of the supernatant was determined via the dinitrosalicylic acid (DNS) colorimetric method (Woolnough *et al.*, 2010). A reagent blank was also run simultaneously in which no food sample was applied.

4.11.1 Reducing sugar analysis of supernatant

Dinitrosalicylic acid reagent was made by dissolving, 10 g 3,5-dinitrosalicylic acid in 400 mL, 2 M NaOH with warming and vigorous stirring, and 300 g sodium potassium tartrate tetrahydrate in 500 mL distilled water. The two solutions were mixed together and made to 1 L with distilled water. Acetate Buffer, 0.1 M pH 5.2, was made by dissolving 13.6 g sodium acetate trihydrate in 900 mL distilled water. This was adjusted to pH 5.2 with 0.1 M acetic acid, 4 mL 1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were added and then it was made to 1 L. Enzyme mixture A, was made using amyloglucosidase (EC 3.2.1.3. from *A.niger*, (Megazyme, E-AMGDF; 3260 U/mL)) and invertase (EC 3.2.1.26 from yeast (Megazyme, E-INVRT; 2000 U/mL)) both 1 % in 0.1 M acetate buffer pH 5.2.

An aliquot of 0.05 mL of each sample in ethanol was placed in a tube with 0.25 mL enzyme solution A. For each run a sample blank was also run with 0.05 mL of distilled water instead of the sample. The tubes were then agitated and rested for 10 min. at room temperature to allow any incompletely hydrolysed starch fragments to be broken down into measurable glucose. Then 0.75 mL DNS mixture (0.5 mg/mL glucose: 4M NaOH: DNS reagent mixed in ratio 1:1:5) was added to each tube. The tubes were covered with foil and heated at 95-100 °C in a boiling water bath for 15 min. Following heating the samples containing reagent blanks, glucose standards and test samples were cooled and diluted with 4 mL of water before transferring to cuvettes (**Figure 4.5**). The absorbance value for each tube was measured at 530 nm wavelength.

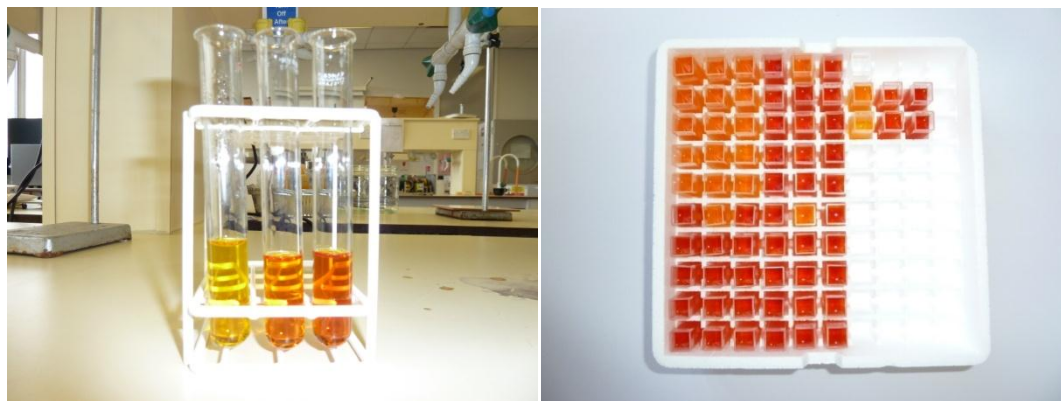


Figure 4.5. DNS procedure, to measure glucose produced, showing different colour responses due to varying amounts of glucose present.

4.12 Sensory analysis

Preliminary sensory analysis was carried out on 4 samples out of the 23 products developed. The aim of this preliminary sensory study was to compare attributes of hardness, crispiness, mouth feel and acceptability, sensorially, between the different samples, and instrumentally (using the data acquired from the texture analysis results). Volunteers were recruited from Hollings Faculty, Manchester Metropolitan University to conduct the sensory trial. Samples were given random numbers and presented in random order, each attribute was scored by marking one box in a line of 11 across the page (example provided in appendix ‘Sensory response sheet 1’). Hardness was ranked hard to soft, crispiness low to high, mouth feel, unpleasant to pleasant and acceptability low to high. In total 50 participants took part in the evaluation. The data was analysed using Fizz software (Biosystemes Couternon, France).

4.13 Statistical analysis and data evaluation

Unless otherwise stated all determinations were made in duplicate, and mean \pm standard deviation (SD) values are presented. Excel (Microsoft Corporation, USA) was used to carry out two tailed t-tests where appropriate to establish *P* values. All analyses of the phase one experiment were conducted comparing the test samples against the control. *P* values are noted where $P \leq 0.05$. Minitab 16 (Minitab Pty. Ltd. Sydney) was used to calculate Pearson’s correlation there was no controlling for confounders.

Chapter 5

Extrusion Processing, Physicochemical Analysis and Nutritional Methodology Used for Evaluation of the Effect of Selected Dietary Fibres into Extruded Snack Products and Their Use in a Clinical Trial; Phase Two.

5.1 Rationale

Following the screening of 8 dietary fibres in **section 3.2**. Two dietary fibres were selected for further analysis in terms of physicochemical and nutritional evaluation. Psyllium and oat bran were selected as dietary fibres showing the greatest possibility of incorporation into a snack food product. This was based on the expansion, texture and *in vitro* digestibility results obtained following the analysis conducted using the methodology in *Chapter 4*. A level of inclusion of 15 % was chosen in order to achieve a dose rate of 15 g of dietary fibre per 100 g of serving of the food product.

The 15 % psyllium and oat bran snack products were manufactured as described below, **section 5.2** and the expansion ratio, texture, fibre, and product pasting properties were evaluated using the methodologies previously outlined in *Chapter 4*. A comparison between the *in vitro* starch (**section 4.11**) degradation/glucose release of the products to the *in vivo* glucose response was conducted as well as a sensory evaluation of the products.

The aim of this part of the study was to investigate the potential acceptance of fibre rich food products by the consumer, and the efficacy of fibre addition in terms of manipulating human glucose response compared to *in vitro* glucose release analysis. For convenience only methodologies not previously used and where adaptations were made to the protocol are mentioned in this section.

5.2 Extrusion process of snack products used in stage two of the research study

Snack products, consisting of a control, 15 % psyllium and 15 % oat bran, were produced using the recipe given in **Table 3.1** and the same extruder set point values (**section 3.4**) variable parameters (**Table 11.2** appendix).

However as a point of deference, after extrusion the collets were allowed to dry and cool for one hour at ambient temperature before being toasted in the oven at 140 °C for 10 minutes. This procedure was adopted after an exchange of discussions with an extruder manufacturer attending an AACC extrusion technology conference. The reason for the slight change in procedure was to improve the texture (mouth feel) of the samples. The collets were allowed to cool completely before being sealed into air tight bags.

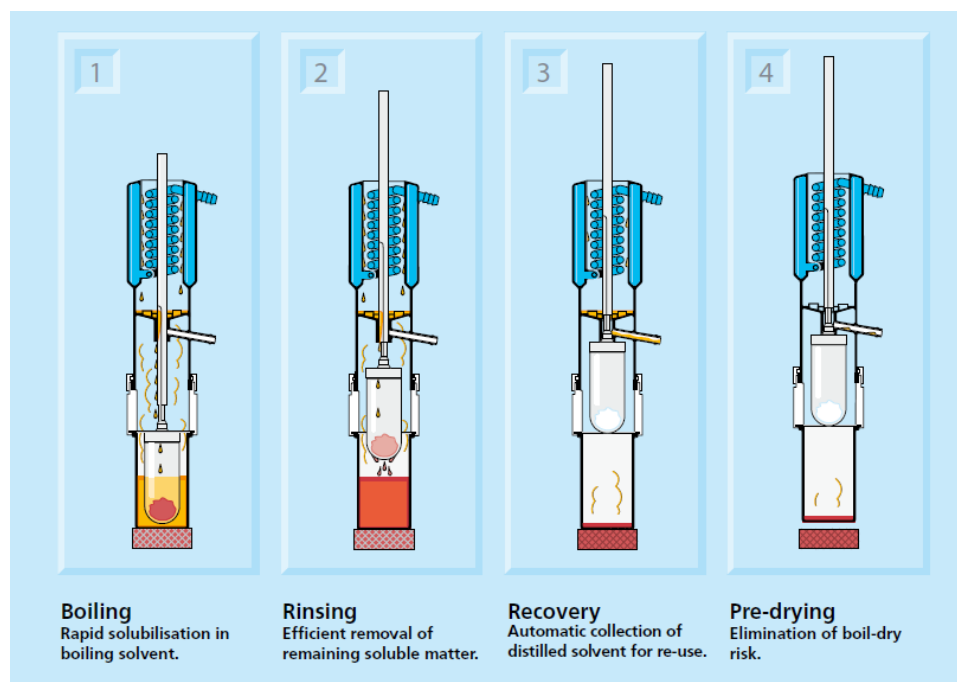
5.3 Fat analysis

The fat content of the samples manufactured in **section 5.2** were determined in order to obtain a compositional analysis of the samples and understand the potential effects of fat on nutrient availability. The Soxtec method utilising solvent extraction in a Soxtec HT 1043 extraction unit was used to determine percentage fat (**Figure 5.1**). Approximately 2.5 g of milled sample was weighed into a paper thimble and a collection cup was pre-tared. The sample was boiled in petroleum ether before being rinsed, then the petroleum ether was evaporated and recovered for subsequent use. The collection cup was reweighed giving the total amount of fat in the sample.

5.4 Protein determination using the Kjeldahl method

Approximately 1 g sample was accurately weighed into a digestion tube. Two Kjeldahl tablets (each containing 3.5g K₂ SO₄ and 0.0035g Se) and 15 mL concentrated sulphuric acid were added. The tube was heated in a digestion block to 420 °C until the solution became clear and removed from the heat after a further 20 min. The tube was cooled, and approximately 70 mL hot distilled water were added, before it was placed in a distillation unit. A conical flask containing 25 mL of 4 % boric acid solution (containing indicator) was placed under the condenser outlet. 30 mL of 40 % NaOH was dispensed in to the

digestion tube and distillation lasted for 4 min. The resulting ammonium borate solution was titrated with 0.1 M hydrochloric acid, to a mauve/grey end point.



http://www.foss.dk/~media/Files/Documents/Industry%20solution%20documents/Brochures%20and%20data%20sheet/Soxtec/Soxtec%20brochure_GB.ashx (accessed 29/11/11)

Figure 5.1. Illustration of the steps involved in the automated fat determination process of the Soxtec method.

5.5 Ash determination

Approximately 4g of sample was weighed into a clean, dry and tared crucible. The crucible was placed in an oven at 565 °C for 5 hours, the crucible was reweighed and percentage ash calculated.

5.6 Carbohydrate determination for *in vivo* study

Percentage moisture and fibre were determined as described in **sections 4.1** and **4.4** respectively. Percentage fat, protein and ash were determined as described in **sections 5.3**, **5.4** and **5.5** respectively. Carbohydrate was determined by difference; it was assumed that the remaining percentage of product after taking into account ash, moisture, protein, fibre

and fat was carbohydrate. This value was used to calculate a standard portion size of carbohydrate given to participants in the study.

5.7 Sensory analysis

Sensory analysis was carried out on the snacks produced for the *in vivo* study by recruiting departmental staff and students to taste the product. A non-trained panel was used and the methods followed general methods used to determine hedonic rating of the samples. All participants were provided with information about the project and gave written consent (information sheet 1, and consent form 1, see appendix). The three snacks were each given a random number and presented in a random order (using a sensory computer programme to generate the numbers and the random orders for 50 participants). The snacks were placed in individual containers and presented to panellists on a tray (**Figure 5.2**).



Figure 5.2. Trays with collets ready for sensory analysis

Individual sensory analysis booths with controlled lighting and air flow were used to avoid outside influences impacting on the perception of product quality (**Figure 5.3**). Participants were asked to assess the attributes of appearance, smell, flavour, texture, crunchiness, after taste and over all acceptability on a linear scale of 1-10, one being highly unacceptable and 10 being highly acceptable. Results were statistically analysed using Fizz software (Biosystemes Couternon, France).



Figure 5.3. Showing sensory booths, from the administration side and participant side

5.8 Intervention study to evaluate *in vivo* glucose response following ingestion of test snack products

The design used for the intervention study was a randomised crossover study carried out to compare the blood glucose response of the three snacks, control, psyllium 15 % and oat bran 15 %. Twelve healthy volunteers were recruited via the Manchester Metropolitan University (MMU) staff website (4 male and 8 female). The study was approved by the MMU Hollings Faculty Ethics Committee (approval form 1, appendix), informed written consent (information sheet 2 and consent form 2, appendix) was obtained from all participants. Before taking part in the main trial a pre-study screening questionnaire was completed (questionnaire 1, appendix).

After pre-screening 14 volunteers, two participants were released from the trial on their first session either due to unforeseen circumstances or an inability to draw sufficient blood through a finger prick. A further two of the participants consumed the three snacks but not the drink as due to work commitments they were unable to attend a final session before the trial ended, their data was included in the study.

All volunteers used for the intervention study were healthy individuals between 18 and 40 years of age, non-smokers, free of metabolic disorders and had a body mass index of 22.5-28. The ethnicity of the subject group was 11 Caucasian and 1 East Asian. None of the subjects were taking any medication that would interfere with glucose metabolism.

Volunteers were asked to consume a snack standardised to contain 25 g carbohydrate (or a standard drink with 25 g glucose). The snacks were provided in a randomised order. Each participant was requested to fast for 12 hours (overnight) prior to attending the study as per

standard practice (Kendall *et al.*, 2008). Each participant attended four times to consume either a snack or the standard drink. On arrival for the initial visit the participant was weighed and measured to confirm their BMI. Blood glucose was tested on every arrival to establish a baseline (fasting blood glucose). The subjects were then asked to consume their allocated snack within 10 minutes (with 250ml water available). After consuming the snack or drink blood glucose was tested at 20, 60 and 120 min. During the trial subjects were offered water to drink. The amount of water consumed by each subject was recorded to be consistent between trial dates. After the final blood test participants were offered a snack before they left.

5.8.1 Blood glucose analysis.

A qualified phlebotomist took blood samples using a finger prick method of blood retrieval. Each participant provided written consent (consent form 3, see appendix). Each blood sample (30- 50 μL) was collected in a new heparinised capillary tube (**Figure 5.4**).

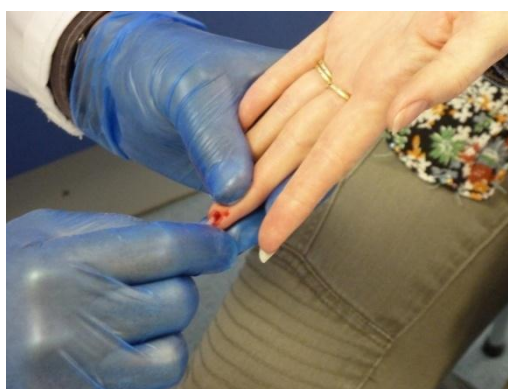


Figure 5.4. Collection of blood into heparinised tube.

The blood was mixed with the heparin to prevent clotting then analysed for capillary blood glucose levels, a pipette was used to transfer a 20 μL blood sample for analysis (**Figure 5.5a.**). Glucose analysis was carried out using an Analox GM 7 microstat (Analox Instruments, London) as illustrated in **Figure 5.5b.**



Figure 5.5. a) transfer from capillary tube to pipette b) placement in Analox microstat.

5.9 Determination of Area Under the Curve

Glycaemic response to a snack product was recorded as the area under the curve (AUC). Fasting blood glucose (FBG) was taken as a baseline, subsequent measurements were calculated as a difference from FBG to give a glycaemic response. Glycaemic response is plotted against time giving a glycaemic response curve. To be able to calculate the area between the response curve and the x axis it can be divided into trapezoids. The area of each trapezoid can be found using the equation known as the trapezoid rule $0.5 \times (\text{time}_2 - \text{time}_1) \times (\text{height}_1 + \text{height}_2)$, then all are added together. This can be summarised as:-

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i)(y_i + y_{i+1})$$

where there are $n+1$ measurements y_i at times t_i ($i = 0, \dots, n$) (Matthews *et al.*, 1990). The area can be divided by the length of time the measurements were recorded over in order to standardise the values.

5.10 Statistical analysis and data evaluation

Unless otherwise stated all determinations were made in duplicate, and mean \pm standard deviation (SD) values are presented. The phase two snacks, psyllium and oat bran and a control sample, were tested against each other for statistical differences using Excel (Microsoft Corporation, USA) two tailed t-tests to establish P values. Minitab 16 (Minitab Pty. Ltd. Sydney) was used to calculate Pearson's correlation there was no controlling for confounders.

Chapter 6

Results and Discussion of Phase One

6.1 Introduction

This chapter covers the preliminary extrusion run. The results are presented and briefly discussed.

Figure 6.1. Showing collets produced. **Figure 6.1** illustrates the collets produced from the extrusion run. The image illustrates some of the differences in appearance of the collets. In particular, the production run for the sample containing 15 % mushroom waste proved to be difficult and yielded inconsistent results. **Figure 6.1** illustrates that these samples were considerably different in appearance to the others. In addition it was not possible to collect enough consistent sample from this set of collets to be able to conduct all analyses. Although the results from these collets are presented in most analysis I would suggest that those analyses that were carried out would not necessarily be reproducible due to the need to rapidly alter feed rate, water rate and screw speed in order to keep the extruder functioning safely.



Figure 6.1. Showing collets produced.

6.2 Percentage moisture loss

Figure 6.2 illustrates the percentage moisture loss obtained during the extrusion process. Actual moisture percentages recorded in **Table 11.3** (appendix). It can be seen that samples containing β -glucan and psyllium, (excepting the 15 % level sample), showed greater percentage moisture loss compared to the control sample. This could be due to the β -glucan and psyllium absorbing high levels of moisture which was bound less tightly during the extrusion process than to other fibres. The inclusion of oat bran, mushroom waste, gatafolia and super gum did not significantly alter the moisture loss of the extrudates during extrusion. Inclusion of inulin at 5 % and 10 % levels reduced moisture loss of extrudates. This may be due to the high degree of solubility associated with fibre components from inulin.

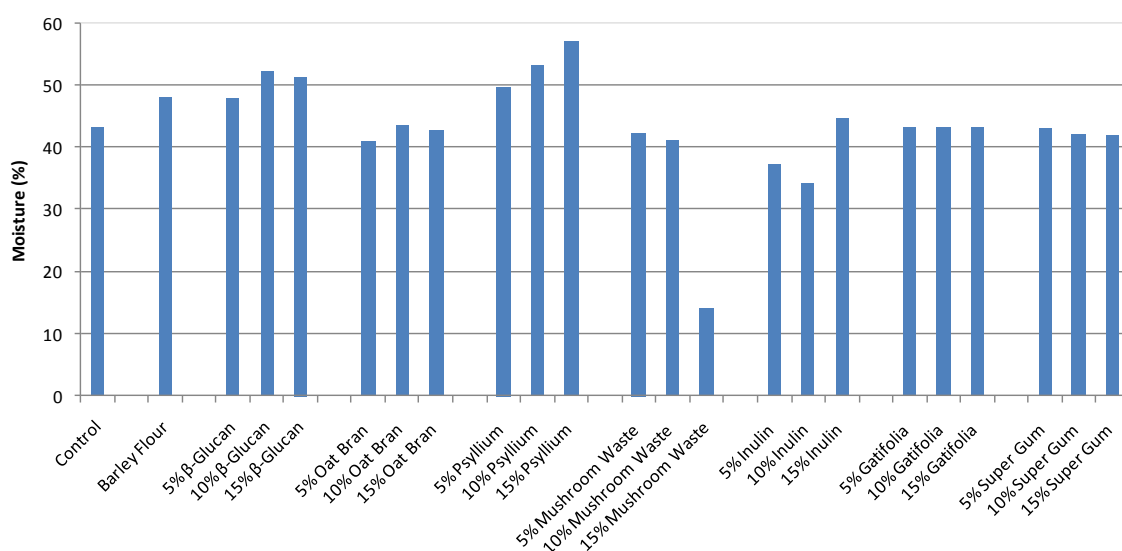


Figure 6.2. Percentage moisture loss as a result of processing (n=2)

6.3 Starch

Starch was not significantly different in raw samples at 5 % inclusion (**Figure 6.3a**). It is possible that this is indicative of sample error in the analytical procedure so that any potential variation at such a relatively low level is not statistically evident. There is a general (non-significant) trend that inclusion at 5 % level did lower the apparent starch content of the raw samples. In the extruded samples the inclusion of β -glucan, oat bran, psyllium, inulin, gatafolia and super gum all resulted in a reduction of apparent starch content. One would expect that by substituting 5 % of flour with a mainly non-starch based

product (such as β -glucan, oat bran, psyllium, inulin, gatafolia and super gum) that the apparent starch content should reduce by between 4-5 %. It is interesting to note that this observation is not statistically viable in the raw samples.

At 10 % inclusion (**Figure 6.3b**) all samples in raw and extruded state (excepting the extruded oat bran sample) were significant different from the control ($P \leq 0.05$). There is a trend that the reduction is within the 10 % range expected from direct substitution.

At 15 % inclusion in the raw state, all samples (except oat bran) were significantly different ($P \leq 0.05$) to the control. In the extruded samples all samples except oat bran were significantly different ($P \leq 0.05$) to the control (**Figure 6.3c**).

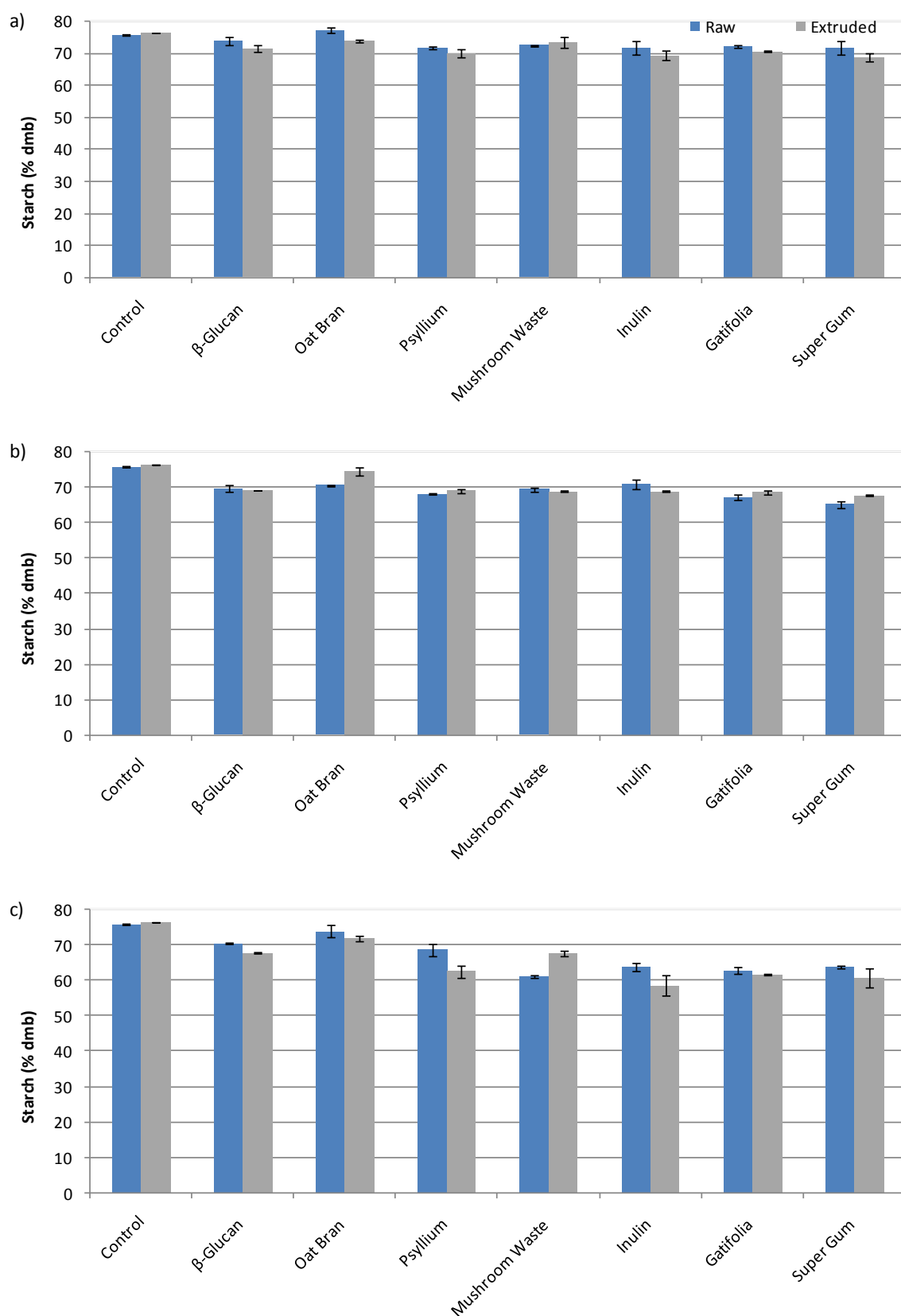


Figure 6.3. Percentage starch on a dry matter basis comparing raw and extruded product at a) 5 %, b) 10 % and c) 15 % inclusion rates (n=2) (data expressed as mean values \pm standard deviation).

6.4 β -Glucan

Figure 6.4 shows that percentage β -glucan was significantly different at 5 % inclusion only the inclusion β -glucan and oat bran were significantly different ($P \leq 0.05$) to the control. This observation was also similar in the extruded products. Similarly at 10 % and 15 % inclusion only oat bran and β -glucan were significantly different ($P \leq 0.05$) to the control in both raw and extruded samples (**Figure 6.4 b and c**).

This result is to be anticipated as the β -glucan and oat ingredients should be rich in β -glucan material. In both cases the increase in β -glucan content is dose responsive. Again would be expected from a simple substitution point of view.

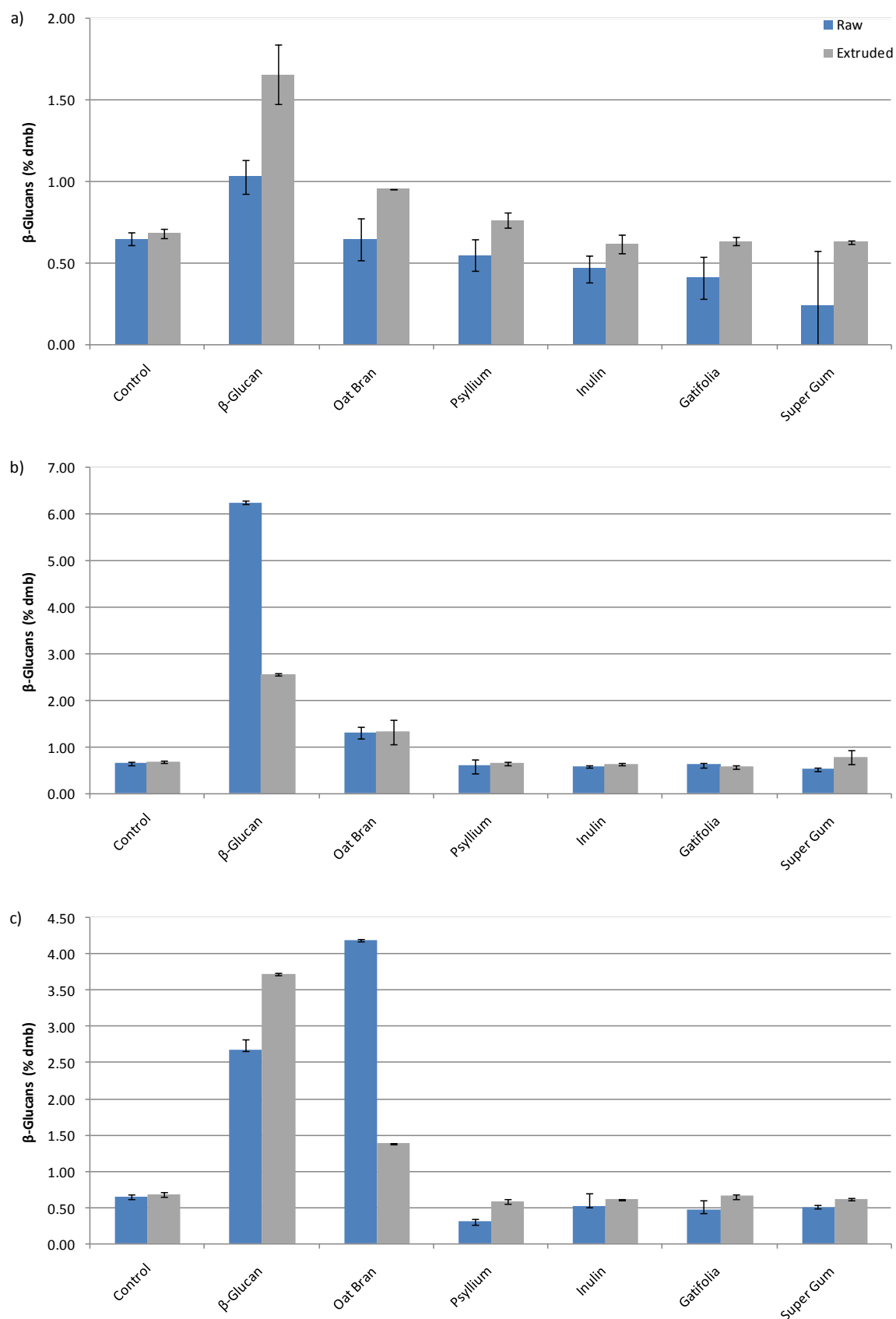


Figure 6.4. Percentage β -glucan on a dry matter basis at a) 5 % b) 10 % c) 15 % inclusion rates (n=2) (data expressed as mean values \pm standard deviation).

6.5 Dietary fibre

The results for insoluble, soluble and total dietary fibre fractions of extruded control, β -glucan, oat bran, psyllium, mushroom waste and inulin products are shown in **Figure 6.5**. Data showing the fibre content of the fibre ingredients is in **Table 11.4** (see appendix)

6.5.1 Insoluble dietary fibre.

Samples containing 5 % added dietary fibre did not significantly differ from the control sample ($P \leq 0.05$). However inclusion of oat bran, psyllium, mushroom waste and inulin at 10 % significantly increased the level of insoluble dietary fibre compared to the control ($P \leq 0.05$). Similarly, all samples (excepting β -glucan) were significantly different ($P \leq 0.05$) to the control at the 15 % inclusion rate. This indicates a significant content of insoluble dietary fibre in all ingredients other than the β -glucan.

6.5.2 Soluble dietary fibre.

Products containing oat bran and inulin at inclusions at 5 % were significantly different ($P \leq 0.05$) to the control in terms of soluble dietary fibre, creating an increase in soluble fibre content. At 10 % inclusion rate all the fibre rich samples were significantly different ($P \leq 0.05$) to the control. This was similar at 15 % inclusion.

6.5.3 Total dietary fibre.

Total dietary fibre is the combination of both insoluble and soluble fractions. **Figure 6.5** illustrates that at 5 % inclusion rate all samples except β -glucan produced a statistically significant increase in total dietary fibre content compared to the control. At both 10 % and 15 % inclusion rate all samples were significantly different ($P \leq 0.05$) to the control. It is noteworthy to observe that according to this method of fibre analysis both oat bran and psyllium fibre yielded the highest level of fibre increase in the products at the 10 and 15 % levels. This is important to note when considering desirable quality parameters for fibre rich food products.

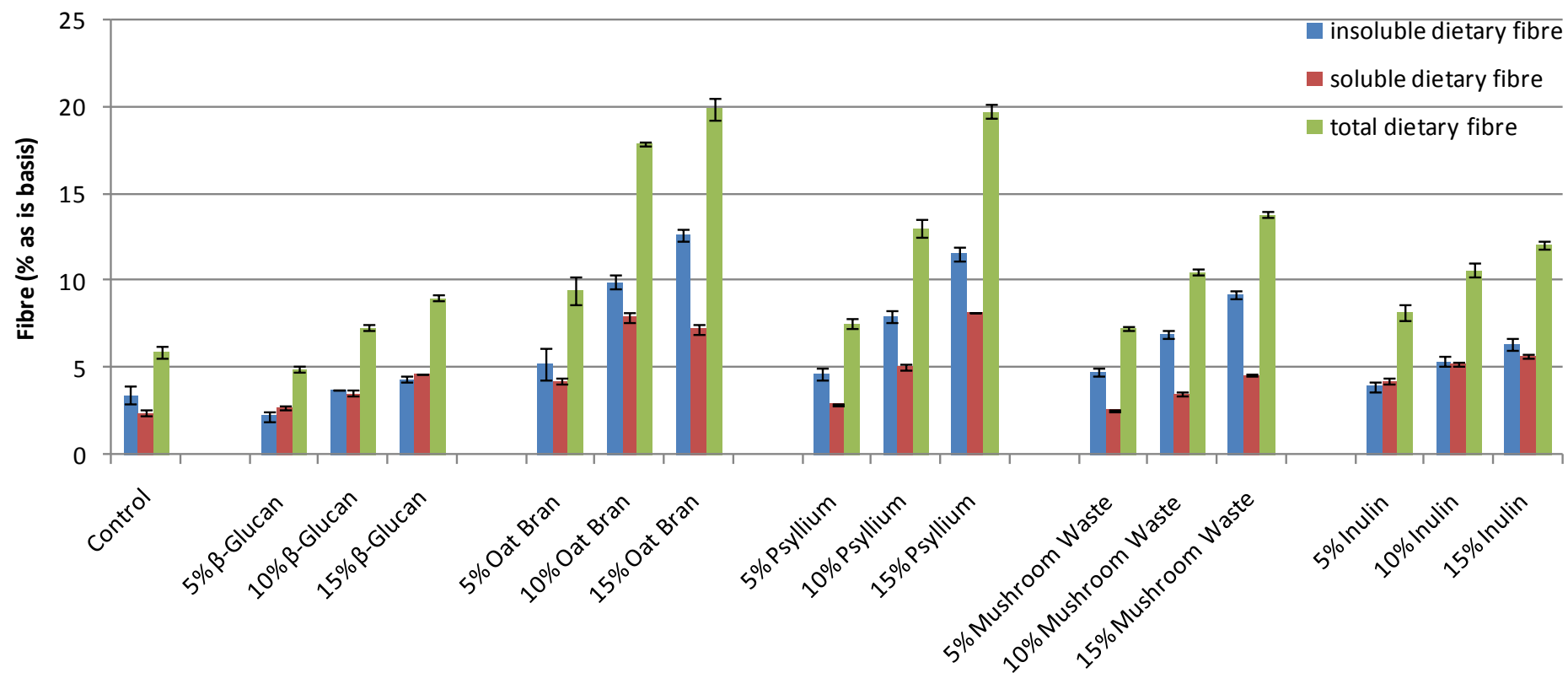


Figure 6.5. Dietary fibre of some samples on an as is basis (n=2) (data expressed as mean values \pm standard deviation).

Table 6.1 shows the results of adding 10 % gatifolia and 10 % super gum, compared with the control they both have a lower fibre content whereas the super gum has a significantly higher amount of soluble fibre than the control, the gatifolia has less soluble fibre than super gum but more than the control. However TDF for gatifolia is similar to the control while super gum has a significant increase compared to the control. Due to the numerous steps of the fibre analysis procedure and consistent complications caused by the nature of these two gums work was concentrated on analysing other fibres at more inclusion rates.

Table 6.1. Dietary fibre content of 10 % Gatifolia and 10 % Super Gum on an as is basis.

	IDF average	SDF average	TDF average
Control	3.47 ±0.53	2.43 ±0.19	5.90 ±0.34
10% Gatifolia	1.42 ±2.01	3.90 ±5.52	5.32 ±3.76
10% Super Gum	1.73 ±2.45	6.45 ±9.12	8.18 ±5.78

6.6 Barley flour: starch, β -glucan and dietary fibre analyses

Results for the product manufactured using 65 % barley flour (total wheat flour replacement) are shown in **Table 6.2** and **Table 6.3** below. The barley flour product had significantly less starch and more β -glucan than the control both in the raw mix and in the extruded product.

Table 6.2. Percentage starch and β -Glucan in the barley flour product on a dry matter basis

	Percentage Starch (dmb)		Percentage β -Glucan (dmb)	
	Raw	Extruded	Raw	Extruded
control	75.75 ±0.24	76.51 ±0.03	0.65 ±0.04	0.68 ±0.03
barley flour	66.94 ±0.54	71.86 ±0.67	3.01 ±0.04	2.40 ±0.05

Compared with the control the barley flour product shows significantly more IDF, SDF and TDF, which is what would be expected as the barley flour is not as refined as the wheat flour.

Table 6.3. Percentage dietary fibre content of the barley flour product on an as is basis

	IDF average	SDF average	TDF average
Control	3.47 ±0.53	2.43 ±0.19	5.90 ±0.34
Barley flour	7.35 ±0.26	4.01 ±0.19	11.36 ±0.07

6.7 Water absorption index

Figure 6.6 shows the WAI of the fibres that were used in the recipe, as well as wheat flour, being the component they were replacing. Mushroom waste, psyllium and β -glucan had a significantly higher ($P \leq 0.05$) WAI than wheat flour, while barley flour, oat bran, inulin, gatifolia and super gum did not appear to be significantly different.

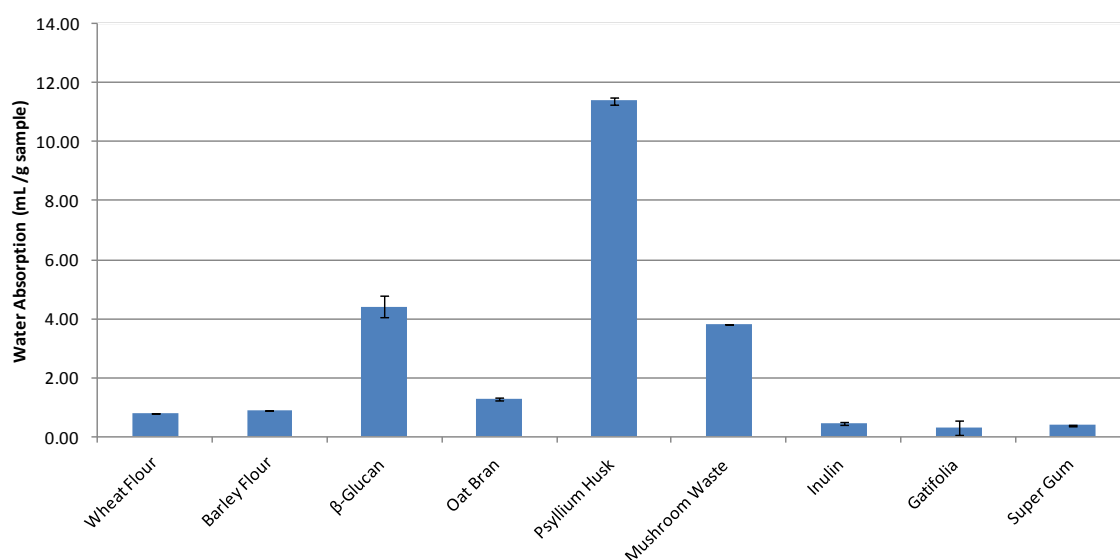


Figure 6.6. Water absorption index of ingredients used in the recipes (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.7 shows the WAI of the recipe mixes before they were extruded. Psyllium at 5 %, 10 % and 15 % and mushroom waste at 10 % and 15 % inclusion rates showed significantly increased ($P \leq 0.05$) WAI compared with the control. Inulin at 5 %, 10 % and

15 % inclusion rates showed significantly decreased ($P \leq 0.05$) water absorption compared to the control.

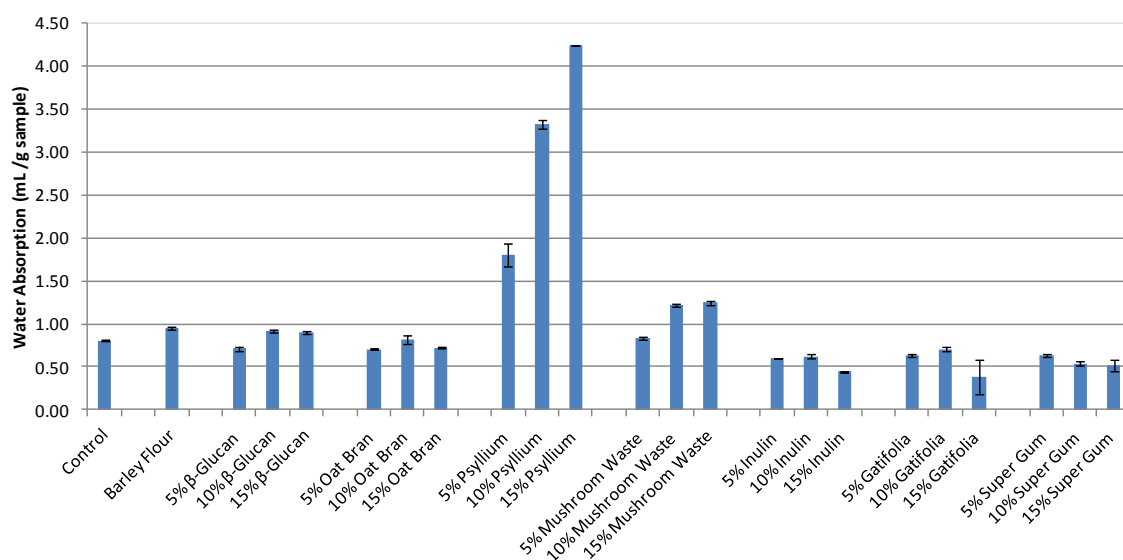


Figure 6.7. Water absorption index of recipe mix before extrusion (n=2) (data expressed as mean values \pm standard deviation).

After extrusion there was a general increase in the WAI of the β -glucan samples, with this becoming significant ($P \leq 0.05$) at the 15 % inclusion rate (**Figure 6.8**). There was a significant decrease ($P \leq 0.05$) in WAI of the sample containing inulin at 15 % whilst other differences do not appear to be significant due to variability of the samples.

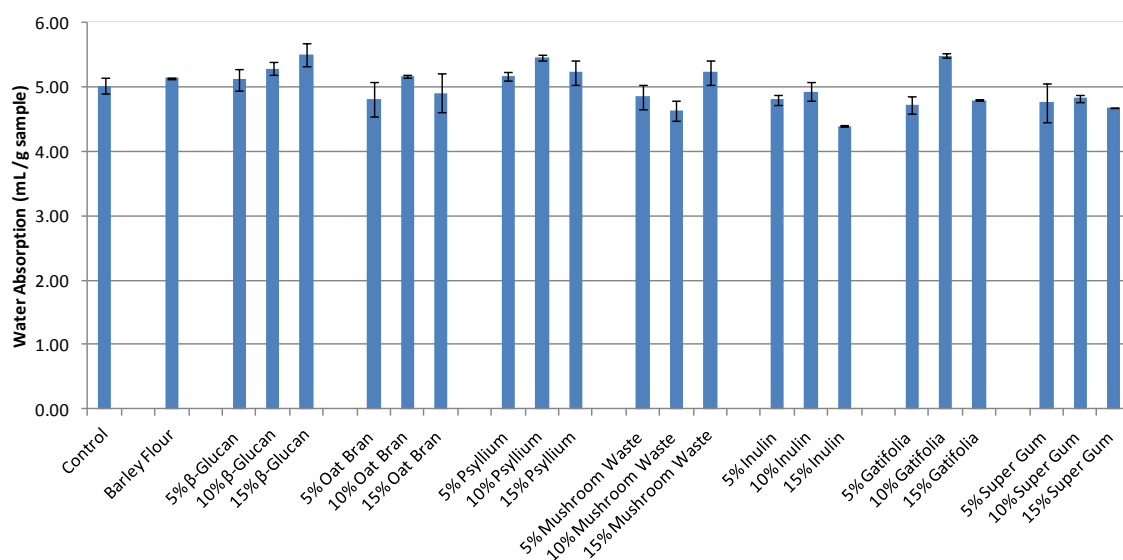


Figure 6.8. Water absorption index of extruded product (n=2) (data expressed as mean values \pm standard deviation).

6.8 Water Solubility Index of ingredients and samples

Figure 6.9 shows WSI of the fibres that were used in the recipe, as well as wheat flour. It is clear from the data that mushroom waste, inulin, gatifolia, super gum and β -glucan all had significantly higher ($P \leq 0.05$) water solubility compared to wheat flour, whereas psyllium had lower solubility compared with wheat flour.

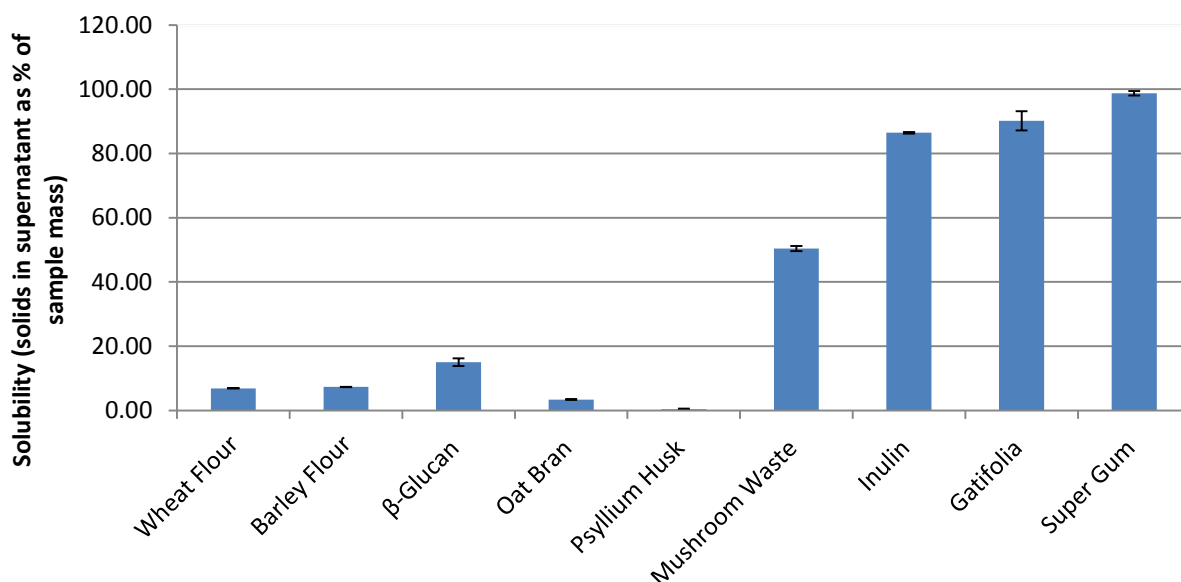


Figure 6.9. Water solubility index of ingredients used in the recipes (n=2) (data expressed as mean values \pm standard deviation).

Water solubility index of the raw and extruded samples was also determined to elucidate the effect extrusion has on this parameter. **Figure 6.10** shows WSI of the raw recipe mix. Oat bran at 5, 10 and 15 % and psyllium at 10 and 15 % inclusion levels showed a significant decrease in WSI of the raw mix compared to the control. Mushroom waste, inulin, gatifolia and super gum inclusions at 5 %, 10 % and 15 % rates showed a significant ($P \leq 0.05$) increase in WSI compared to the control sample. The increase of WSI for these samples was dose responsive; increasing levels of fibre increased WSI of the samples.

Both WAI and WSI are of importance to food manufacture in terms of the amount of water required to be added to a food system to enhance processability, and the viscosity of the product during processing (affecting energy utilisation in many industrial processes). The differences in WAI and WSI illustrate that the food technologists should not assume all fibres behave in the same way during food processing and hence attention is needed to ensure correct water additions are required in order to achieve consistent food products.

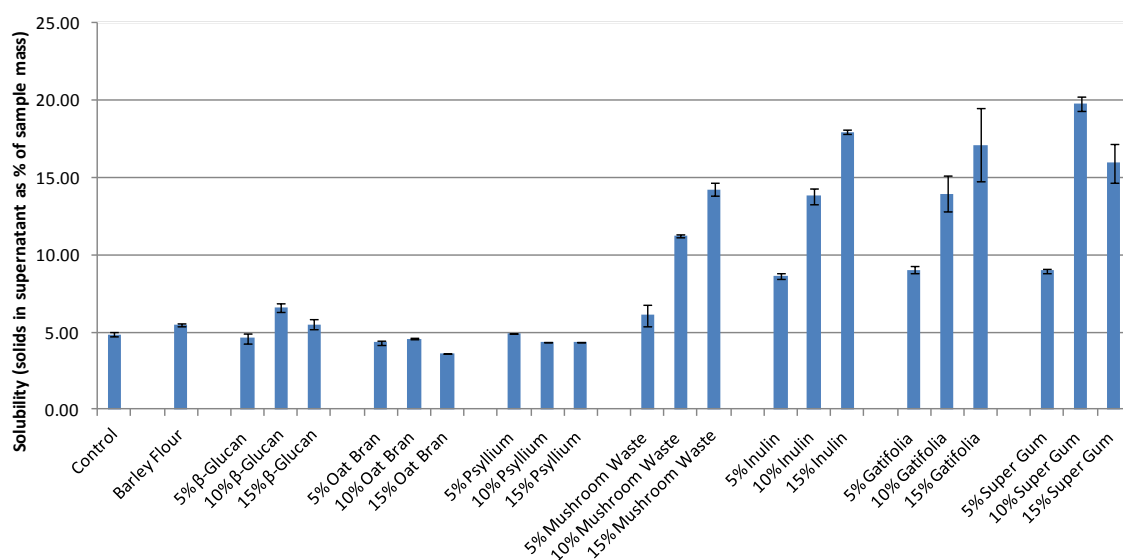


Figure 6.10. Water solubility index of recipe mix before extrusion (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.11 shows WSI of the extruded products, there appears to be a significant decrease between the control and 5 % inulin, 5 % gatifolia, 15 % oat bran and 15 % psyllium samples ($P \leq 0.05$). All samples appeared to be variable which does not allow clear conclusions to be drawn.

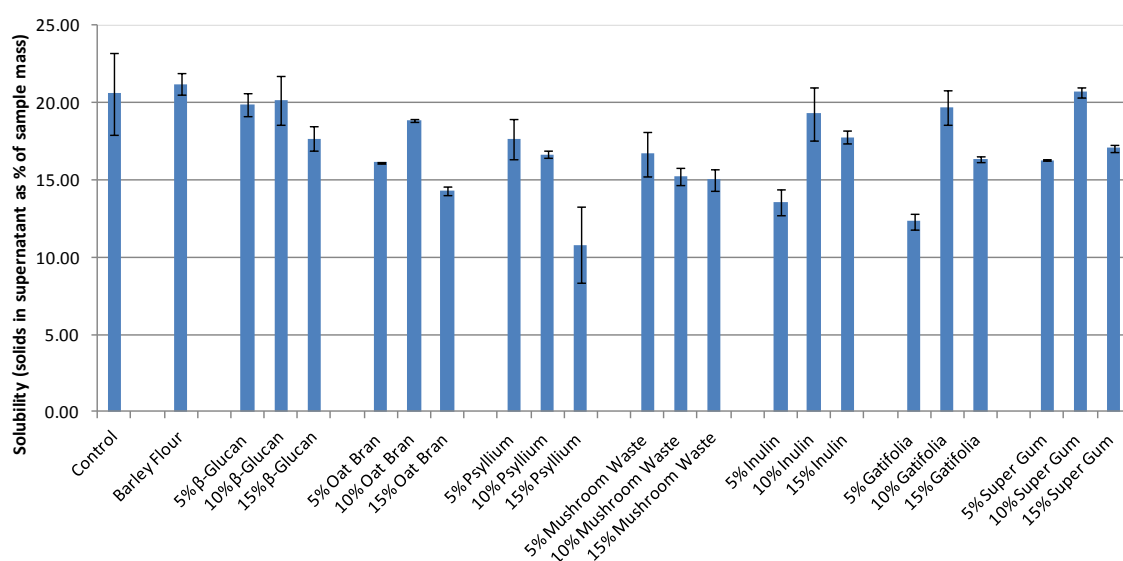


Figure 6.11. Water solubility index of extruded product (n=2) (data expressed as mean values \pm standard deviation).

6.9 Oil absorption index

Figure 6.12 shows the OAI of the fibres that were used in the recipe, as well as wheat flour, mushroom waste, psyllium, β -glucan, barley flour, oat bran, inulin and super gum had a significantly higher ($P \leq 0.05$) OAI than wheat flour.

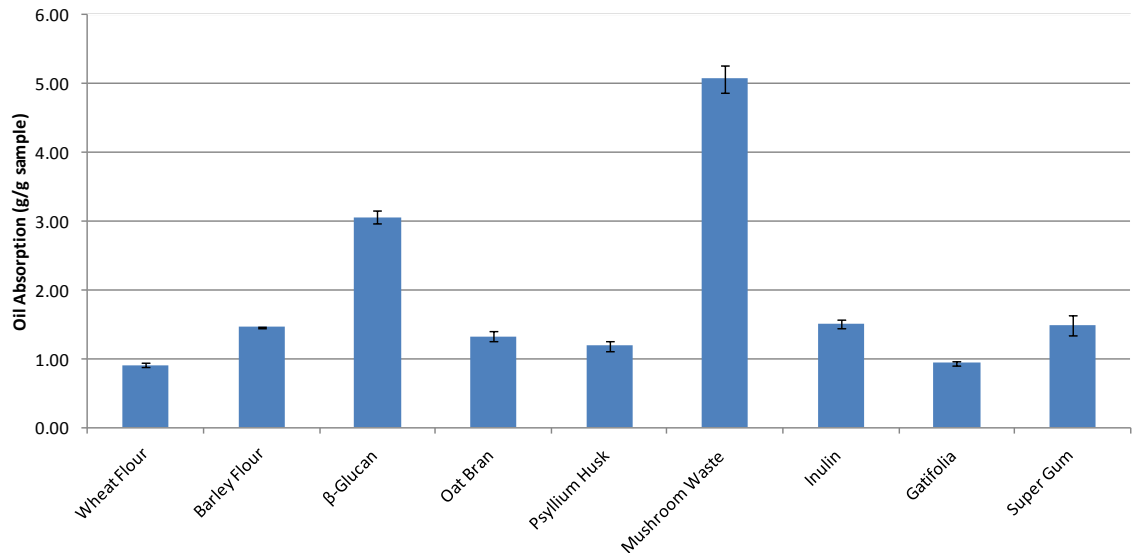


Figure 6.12. Oil absorption index of ingredients used in the recipes (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.13 shows the OAI of recipe mixes before extrusion. Mushroom waste inclusion at 10 % and 15 % significantly ($P \leq 0.05$) increased OAI. Oat bran and psyllium inclusion at 5 % and 15 % significantly ($P \leq 0.05$) decreased OAI sample with 10 % inclusion were too variable for any difference to be significant.

Oil absorption index is important in industrially processing as many flavourings are either oil based or oil is used as a carrier. Thus the food technologist needs to be aware of products with high OAI so as to avoid foods becoming high in fat content or containing excess flavour compounds. Both of these effects would have a negative effect on consumer acceptability of products and also the overall quality of the food manufactured.

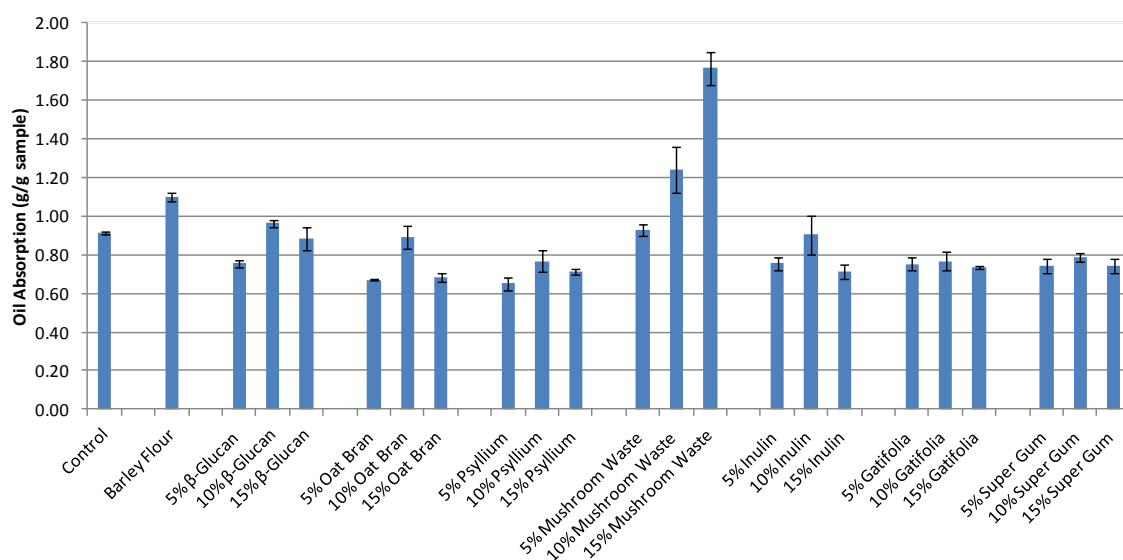


Figure 6.13. Oil absorption index of recipe mix before extrusion (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.14 shows the OAI of extruded product. Compared to the control β -glucan, psyllium, gatifolia at 5 %, 10 %, and 15 % inclusion, oat bran at 5 % and 15 % inclusion and the barley flour sample were all significantly different ($P \leq 0.05$). Any increase appeared not to be dose related. Extrusion did not appear to consistently alter the OAI of samples.

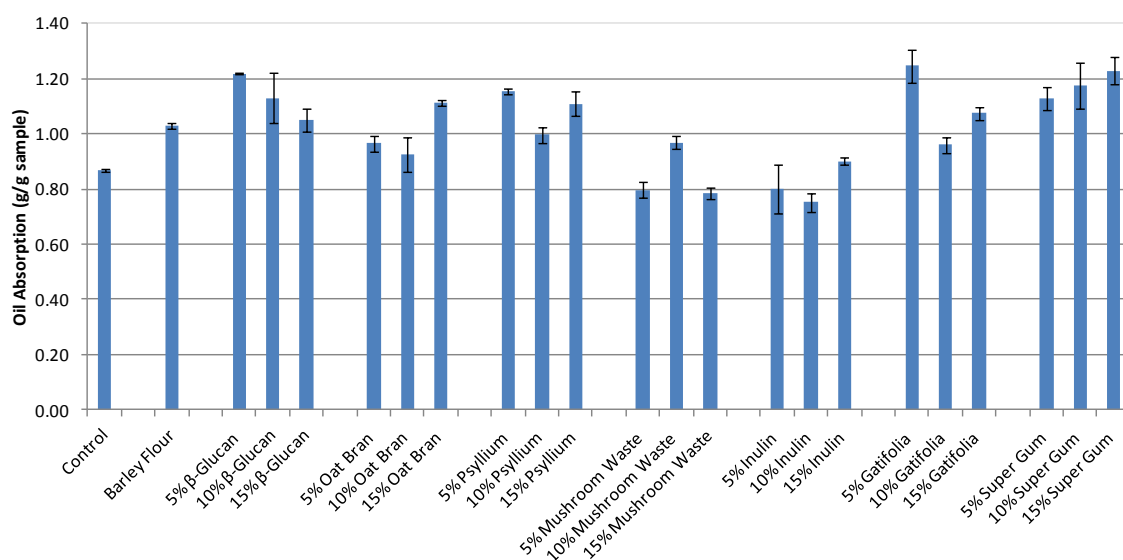


Figure 6.14. Oil absorption index of extruded product (n=2) (data expressed as mean values \pm standard deviation).

6.10 Expansion ratio

The expansion ratios of products were affected by the inclusion of the different fibre ingredients. There were general trends such as an increase in expansion ratio related to the inclusion of β -glucan and psyllium, as well as a reduction in expansion related to the incorporation of barley flour. These were non-significant trends when statistically analysed possibly due to high variability in sample products. This variability of expansion ratio (as observed by some relatively large standard errors) is noteworthy as this would produce difficulties in terms of producing homogeneous samples at a commercial level. However super gum, gatifolia, psyllium and β -glucan at all levels of inclusion and mushroom waste at 15 % all showed a significant ($P \leq 0.05$) increase in expansion ratio compared to the control (**Figure 6.15**).

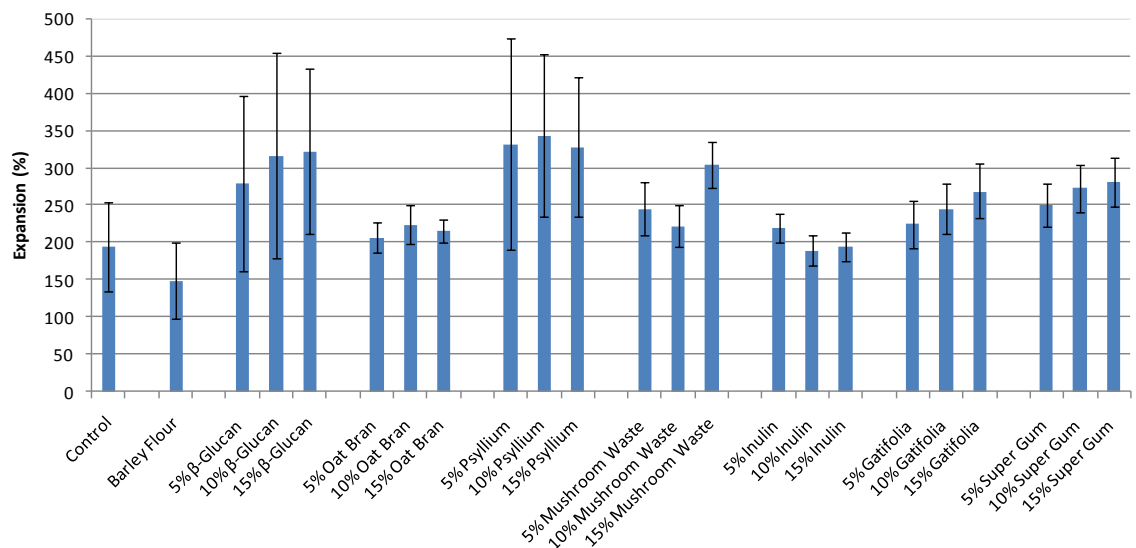


Figure 6.15. Expansion ratio (expressed as a percentage of the die diameter) of extruded products (n=20) (data expressed as mean values \pm standard deviation).

6.11 Density

Inulin, psyllium, oat bran, β -glucan at 5 %, 10 %, and 15 % inclusion, mushroom waste at 10 % inclusion and the barley flour sample all show a significant ($P \leq 0.05$) increase in density compared to the control sample (**Figure 6.16**). There was no clear dose response in terms of fibre levels and density values.

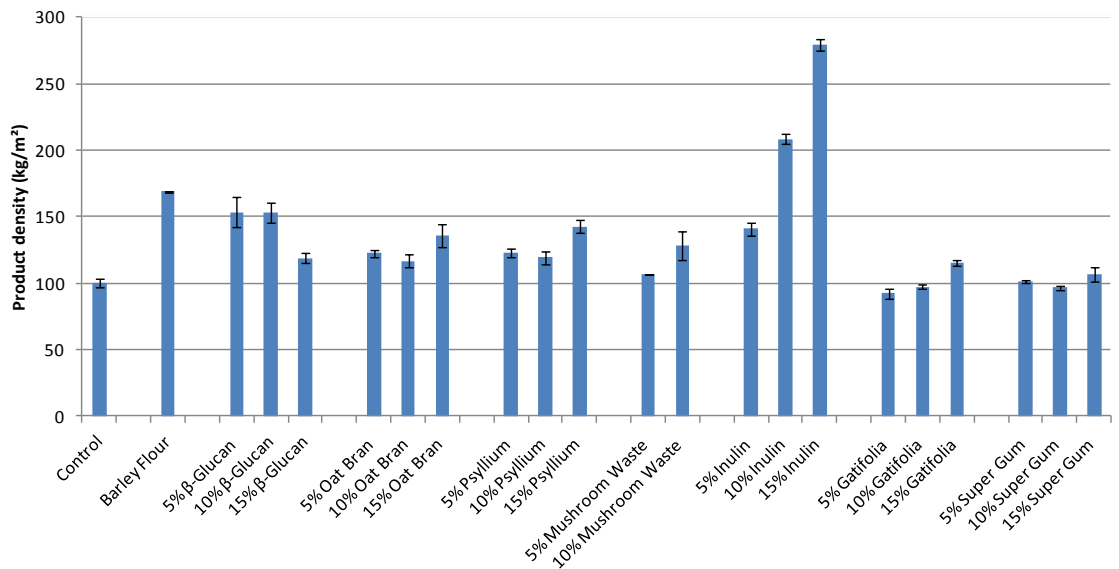


Figure 6.16. Density of extruded products (data expressed as mean values \pm standard deviation).

6.12 Texture

The texture of the extruded products was determined using a Stable Microsystems texture analyser. **Figure 6.17** shows the peak amount of force required to crush the collets produced. Although there is some of variability in the samples these variations tend to be non-significant ($P \leq 0.05$) because of high degree of variability observed in most samples. For instance there is a non-significant trend for an increase in peak force required to crush the product with increasing levels of inclusion of both β -glucan and oat bran, whereas increasing inclusion rates for gatifolia and super gum appeared to reduce the peak force of the samples. Mushroom waste inclusion at 5 and 10 % levels, and inulin, gatifolia and super gum inclusion at 10 % and 15 % levels produced collets which required significantly ($P \leq 0.05$) less force to crush them than the control. It is interesting to note that generally the peak force of all samples was lower than the control.

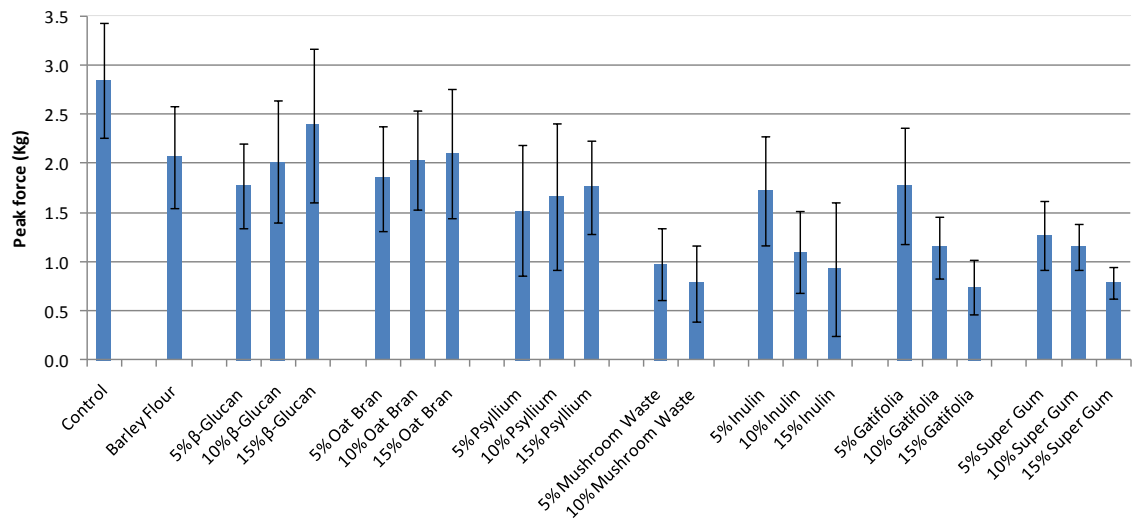


Figure 6.17. Texture determination (peakforce) of extruded products (n=20) (data expressed as mean values \pm standard deviation).

Figure 6.18 shows texture as a measure of the number of peaks requiring 50g force as the probe descended to crush the collet. Oat bran and inulin at all inclusion rate had significantly less peaks than the control ($P \leq 0.05$). Psyllium at all inclusion rates, super gum and β -glucan at 10 % and 15 % inclusion rates had significantly increased peak force requirements ($P \leq 0.05$).

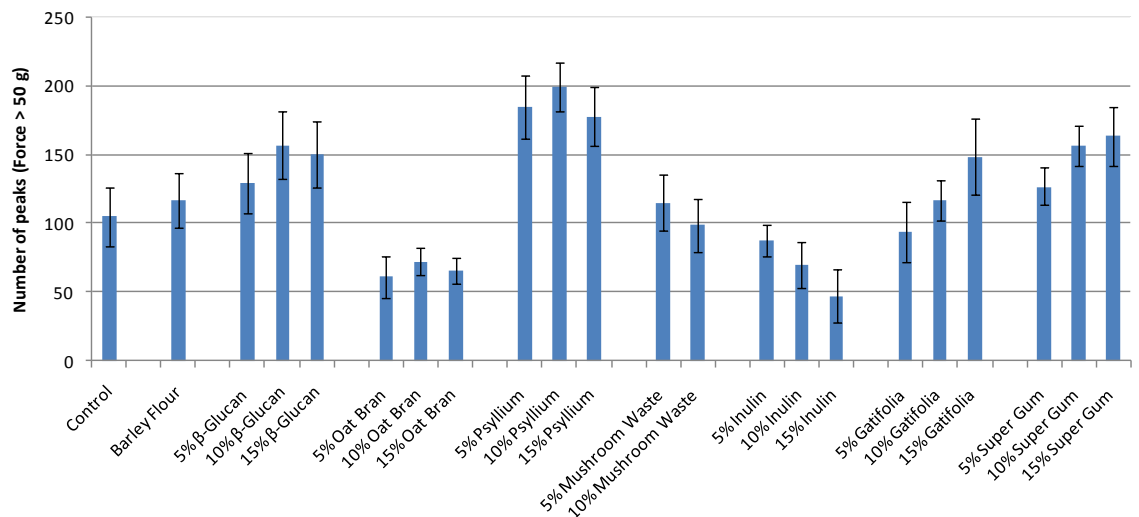


Figure 6.18. Texture number of peaks over 50g (n=20) (data expressed as mean values \pm standard deviation).

Figure 6.19 shows texture with regard to the number of noise events over 20dB. Super gum, β -glucan, psyllium at 5 %, 10 % and 15 % and gatifolia at 10 % and 15 % inclusion

rates showed an increased number of peaks and therefore more fracture events ($P \leq 0.05$). Oat bran at 5 %, 10 % and 15 % and inulin at 10 % and 15 % inclusion rates show a decreased number of peaks hence less fracture events ($P \leq 0.05$).

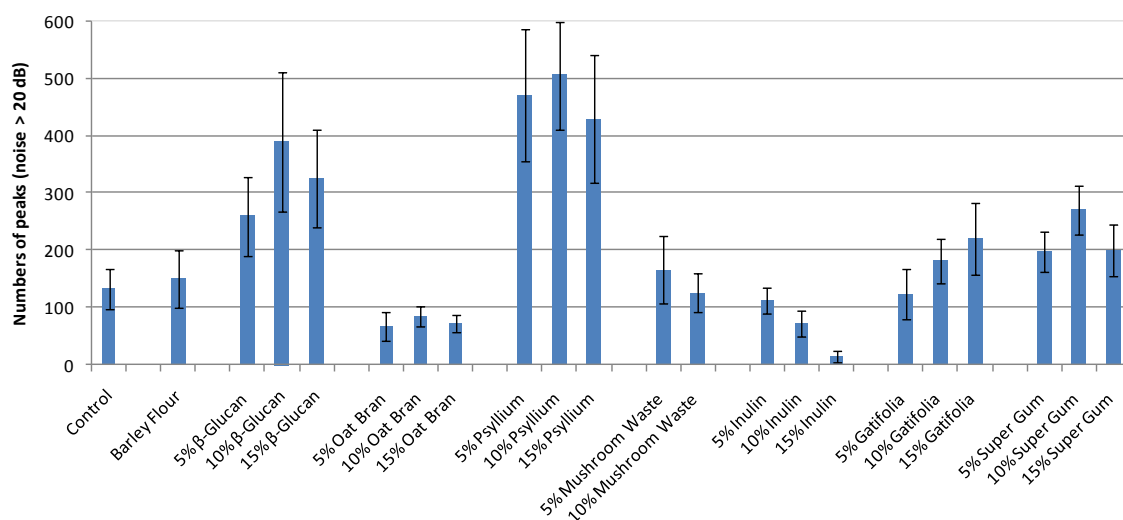


Figure 6.19. Texture number of peaks over 20dB (n=20) (data expressed as mean values \pm standard deviation).

6.13 Preliminary sensory trial.

A preliminary sensory analysis of a selection of samples was conducted to try to determine if a non-trained consumer panel could pick up some of the textural difference observed in the instrumental analysis. Mushroom waste, inulin, gatifolia and super gum were chosen as samples which exhibited well defined differences from the control using the texture analyser. **Figure 6.20** shows a web diagram for selected sensory attributes. The crispiness of products was perceived to be increased with an addition of fibre to the extruded products. This observation did not correlate with the number of peaks determined by force or acoustic measurements but did appear to relate to the overall peak force (**Figure 6.21**). Consumers perceived that the hardness of the products was reduced with fibre inclusion (possibly correlating to the peaks recorded in terms of force and acoustically). Mouth feel was perceived to have been more acceptable in the fibre rich products compared to the control.

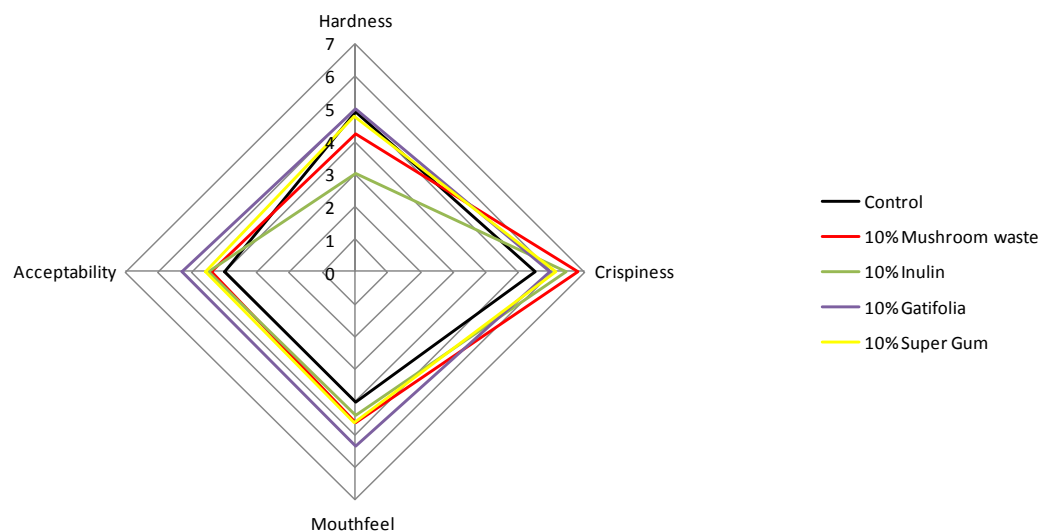


Figure 6.20. Web diagram illustrating selected sensory aspects of snack products.

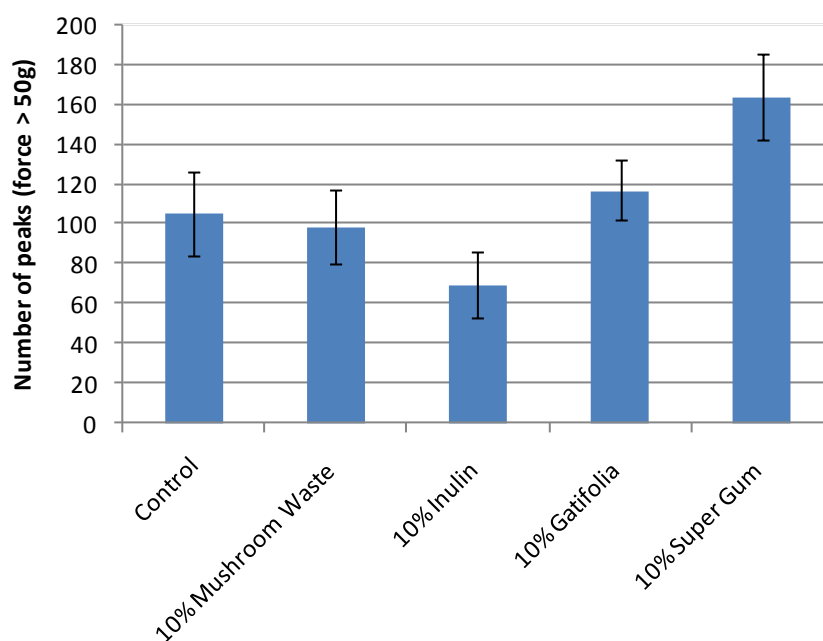


Figure 6.21. Showing number of peaks for products trialled by preliminary taste panel (n=20) (data expressed as mean values \pm standard deviation).

Although not all samples were tested it is possible to see that in spite of perceived differences in terms of hardness and crispiness, consumer acceptability of the products were similar (or improved) when compared against the control product. This was a positive result in terms of being able to produce a high fibre concept product which may meet consumer acceptability.

6.14 Rapid Visco Analyser measurements of raw and extruded products

The thermal pasting properties of both the raw mixes and finished extruded products were determined using a Newport Scientific RVA machine. Although data was recorded in terms of cold peak, peak viscosity, set-back, final viscosity, pasting time and pasting temperature only values for peak viscosity and final viscosity are shown in the following tables in an attempt to condense results.

An exemplar of extruded samples at 5 %, 10 % and 15 % levels are included to show different pasting patterns (**Figure 6.22**). A full range of RVA charts depicting each product at each concentration are provided in the appendix to illustrate general pasting profiles of samples and raw mixes. At 5 % level the pasting profiles of the fibre rich products appear to be higher than that of the control sample. This is reflected also in the 10 % and 15 % level plots. Psyllium, β -glucan, gatifolia and oat bran inclusions significantly increase the pasting profiles compared to the control ($P \leq 0.05$).

Table 6.4 illustrates the peak viscosity and final viscosity values of the sample raw mixes. Barley flour, β -glucan at 10 % and 15 % inclusion, and psyllium at all levels of inclusion produce higher peak viscosity readings compared to the control and also final viscosity readings. Whilst oat bran and mushroom waste inclusion does not affect the peak viscosity values of the raw mixes it does increase their final viscosity readings. Inulin, super gum and gatifolia significantly reduce both the peak viscosity and final viscosity of the raw mixes compared to the control sample ($P \leq 0.05$).

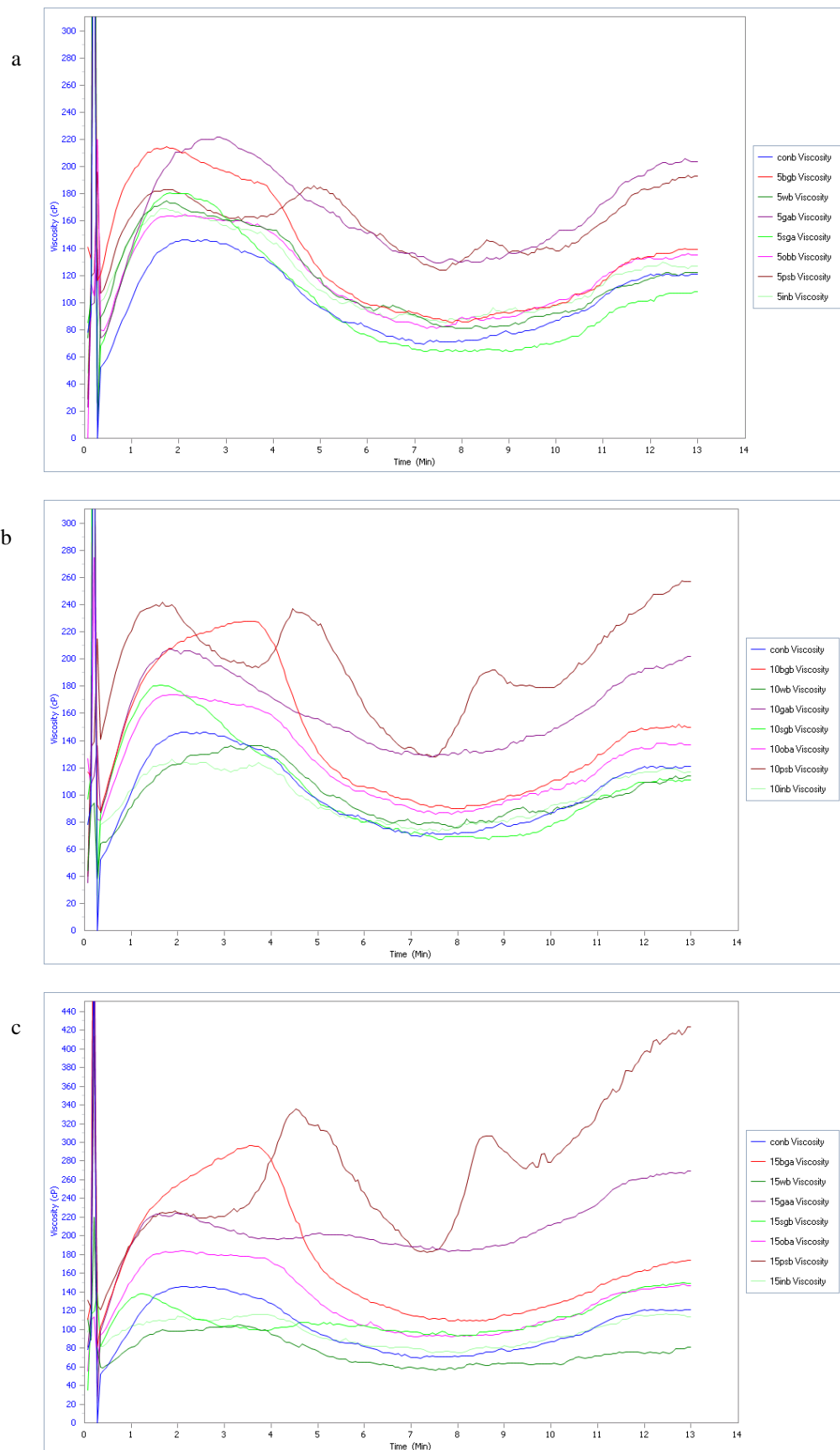


Figure 6.22. Examples of Rapid Visco Analyser pasting profiles of extruded snack products at a) 5 % b) 10 % and c) 15 % inclusion levels.

Table 6.4. Rapid Visco Analyser pasting properties raw snack mixes (data expressed as mean values \pm standard deviation).

Raw material	Peak viscosity (cP)	Final Viscosity (cP)
Control	317.50 \pm 17.68	553.50 \pm 30.41
Barley Flour	643.00 \pm 12.73	1045.00 \pm 24.04
5% β -glucan	315.00 \pm 1.41	622.50 \pm 6.36
10% β -glucan	569.00 \pm 14.14	1203.50 \pm 37.48
15% β -glucan	538.00 \pm 33.94	1125.50 \pm 54.45
5% Oat Bran	311.50 \pm 2.12	572.00 \pm 11.31
10% Oat Bran	325.00 \pm 7.07	582.50 \pm 4.95
15% Oat Bran	365.50 \pm 3.54	656.50 \pm 6.36
5% Psyllium	634.50 \pm 7.78	1031.50 \pm 48.79
10% Psyllium	1207.50 \pm 51.62	1767.00 \pm 22.63
15% Psyllium	1545.50 \pm 132.23	2233.00 \pm 196.58
5% Mushroom Waste	349.50 \pm 17.68	690.50 \pm 20.51
10% Mushroom Waste	366.50 \pm 4.95	754.00 \pm 9.90
15% Mushroom Waste	NA	NA
5% Inulin	218.00 \pm 4.24	421.50 \pm 6.36
10% Inulin	180.50 \pm 17.68	363.50 \pm 19.09
15% Inulin	136.00 \pm 1.41	278.50 \pm 7.78
5% Gatifolia	233.00 \pm 16.97	416.50 \pm 12.02
10% Gatifolia	179.50 \pm 2.12	327.50 \pm 2.12
15% Gatifolia	192.50 \pm 0.71	307.00 \pm 8.49
5% Super Gum	230.50 \pm 0.71	401.00 \pm 14.14
10% Super Gum	139.50 \pm 14.85	265.00 \pm 21.21
15% Super Gum	138.00 \pm 2.83	264.00 \pm 4.24

Table 6.5 illustrates the peak and final viscosity readings of the extruded snack products. The initial observation comparing **Table 6.4** and **Table 6.5** is that extrusion has a significant effect in terms of reducing the peak viscosity of products as well as the final viscosity ($P \leq 0.05$). For instance in the control sample the peak and final viscosity readings of the raw mix samples were 317.50 cp and 553.50 cp respectively. After extrusion these values were reduced to 149.5 cp for peak viscosity and 51cp for final viscosity. This clearly illustrates the effect that extrusion has on the viscous properties of

food samples attributed to the effect of both sheer and cooking during the extrusion process.

Table 6.5. Rapid visco analyser pasting properties of extruded products (data expressed as mean values \pm standard deviation).

Extruded	Peak viscosity (cP)	Final Viscosity (cP)
Control	149.50 \pm 4.95	51.00 \pm 1.41
Barley Flour	189.50 \pm 0.71	46.00 \pm 4.24
5% β -glucan	216.00 \pm 1.41	53.00 \pm 1.41
10% β -glucan	232.00 \pm 5.66	78.00 \pm 25.46
15% β -glucan	297.00 \pm 0.00	65.50 \pm 0.71
5% Oat Bran	171.00 \pm 8.49	53.00 \pm 1.41
10% Oat Bran	173.50 \pm 0.71	51.50 \pm 0.71
15% Oat Bran	187.50 \pm 4.95	56.00 \pm 1.41
5% Psyllium	188.00 \pm 2.83	72.50 \pm 4.95
10% Psyllium	239.00 \pm 4.24	130.50 \pm 2.12
15% Psyllium	344.00 \pm 11.31	238.50 \pm 4.95
5% Mushroom Waste	174.00 \pm 1.41	37.50 \pm 4.95
10% Mushroom Waste	131.50 \pm 6.36	34.00 \pm 5.66
15% Mushroom Waste	106.00 \pm 1.41	25.00 \pm 0.00
5% Inulin	173.50 \pm 6.36	42.00 \pm 0.00
10% Inulin	127.50 \pm 2.12	42.00 \pm 2.83
15% Inulin	122.00 \pm 8.49	38.00 \pm 0.00
5% Gatifolia	216.50 \pm 7.78	74.50 \pm 0.71
10% Gatifolia	220.50 \pm 19.09	78.50 \pm 6.36
15% Gatifolia	228.50 \pm 6.36	88.50 \pm 3.54
5% Super Gum	180.50 \pm 0.71	43.00 \pm 1.41
10% Super Gum	180.00 \pm 1.41	40.50 \pm 4.95
15% Super Gum	145.00 \pm 9.90	57.50 \pm 2.12

Whilst samples containing β -glucan, oat bran, psyllium and mushroom fibre all showed similar response to extrusion (a reduction of about 50 % in terms of peak viscosity values and a reduction by a factor of ten in terms of final viscosity), inulin, gatifolia and super gum samples were less affected by the extrusion process and appeared to retain more of

their peak viscosity values (although final viscosity was affected). This indicates that not all fibres behave similarly in terms of their response to extrusion processing and that potentially the larger molecular weight NSP polymers (β -glucan and psyllium for example) are subjected to greater depolymerisation and shear during the extrusion process than smaller molecular weight polysaccharides such as inulin and super gum.

6.15 *In vitro* starch determination

In vitro digestibility procedures were used to evaluate the degree of susceptibility to starch degradation of both the raw mixes and extruded products. The rate and extent of starch degradation is measured in terms of the release of reducing sugars which in turn can be translated into the release of glucose content over the 120 min digestion process.

Figure 6.23 shows the glucose release over 120 min for β -glucan at 5 %, 10 % and 15 % inclusion rates. It is clear by comparing the graphs a) and b) that extrusion plays a large role in increasing the rate of glucose release of a product ($P \leq 0.05$). For instance there is a trend of starch digestion/glucose release being doubled due to the extrusion process. This may be related to the decrease in peak viscosity as mentioned in the previous chapter.

The rationale for this suggestion being that the major contributing factor to the pasting profile of a carbohydrate rich sample is the gelatinisation process of starch. The extrusion process obviously reduces the potential gelatinisation properties of a starch substance as measured by the RVA. This is actually due to the reduction in polymer conformation due to shear, and also the cooking properties of the extruder increasing gelatinising available starch. This leads to an increase in starch digestibility and hence sugar release as observed by the *in vitro* procedure.

Another interesting observation is that in the raw sample there is no significant difference in glucose release from any of the products. However in the extruded β -glucan samples the amount of glucose released is significantly less ($P \leq 0.05$) than the control at all time intervals except at 0 min clearly indicating a contributing effect fibre has in the manipulation of starch digestibility and hence glucose release from a starch rich product.

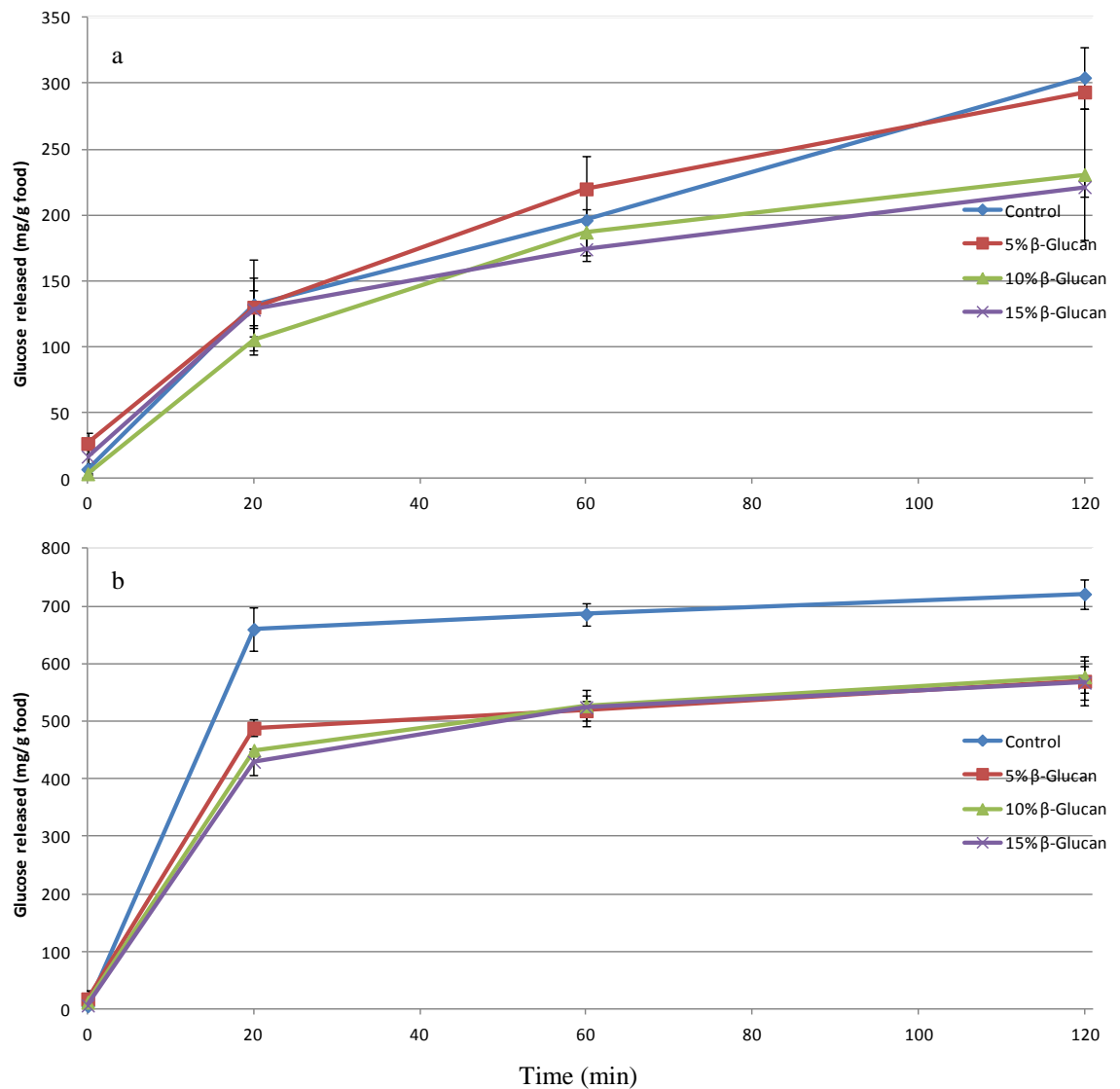


Figure 6.23. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % β-glucan (n=2) (data expressed as mean values ± standard deviation).

The inclusion of oat bran at 5 %, 10 % and 15 % had no significant effect on the glucose released in the raw sample (**Figure 6.24a**). Whilst the digestibility of starch is increased in the extruded samples, inclusion of oat bran at all levels significantly decreased ($P \leq 0.05$) the amount of glucose released is compared to the control at 20 min, 60 min and 120 min (**Figure 6.24b**). Little difference could be observed between the different levels of oat bran inclusion, potentially indicating that the response of fibre in reducing starch digestibility is not necessarily dose related.

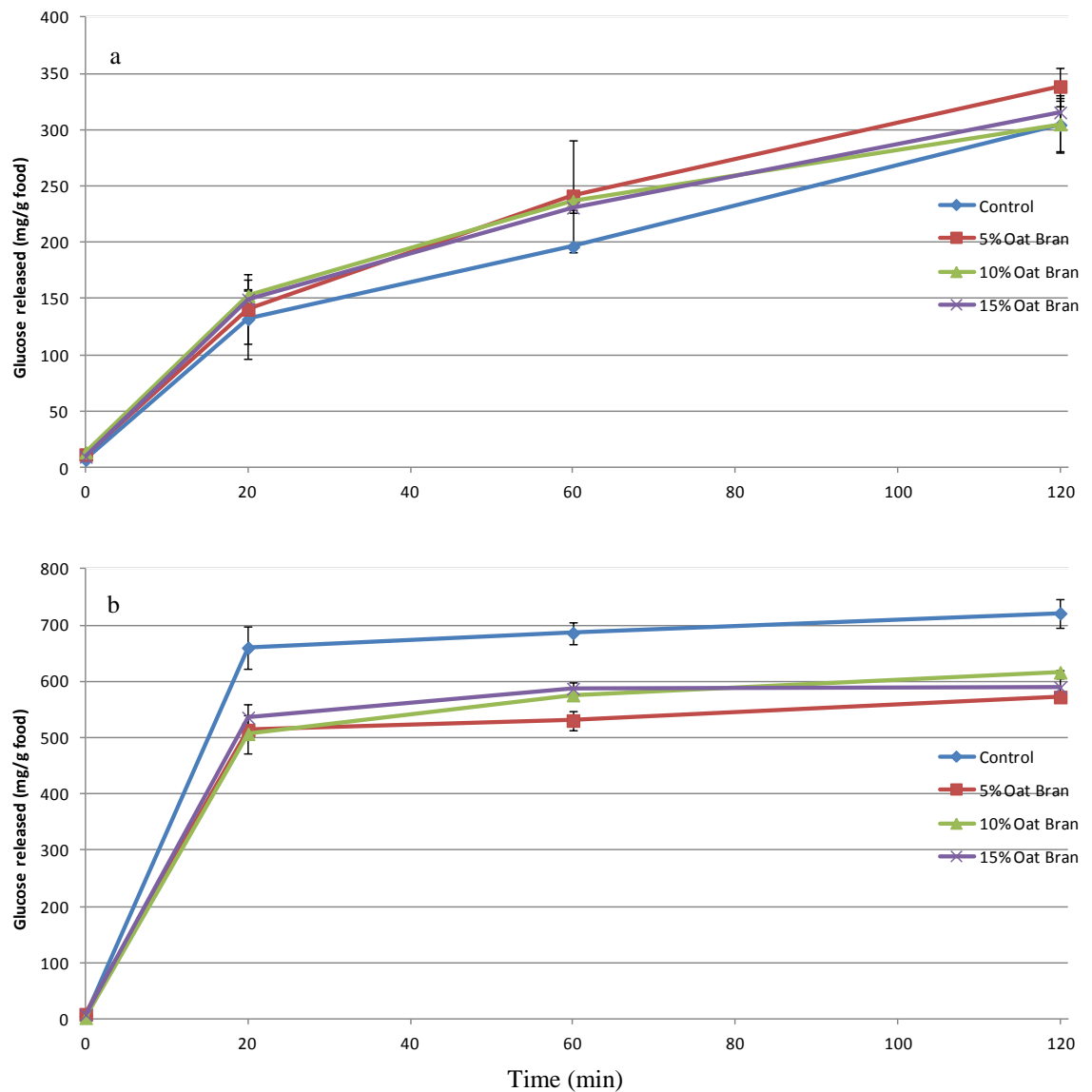


Figure 6.24. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % oat bran (n=2) (data expressed as mean values \pm standard deviation).

In vitro digestion of the raw sample containing psyllium also showed no significant difference in glucose release compared to the control (**Figure 6.25a**). After extrusion samples containing psyllium produced significantly less ($P \leq 0.05$) glucose at 20 min, 60 min and 120 min compared with the control (**Figure 6.25b**). In the case of psyllium inclusion there appeared to be a dose response to reduction in glucose release, in that glucose released was reduced proportionally to the level of ingredient inclusion.

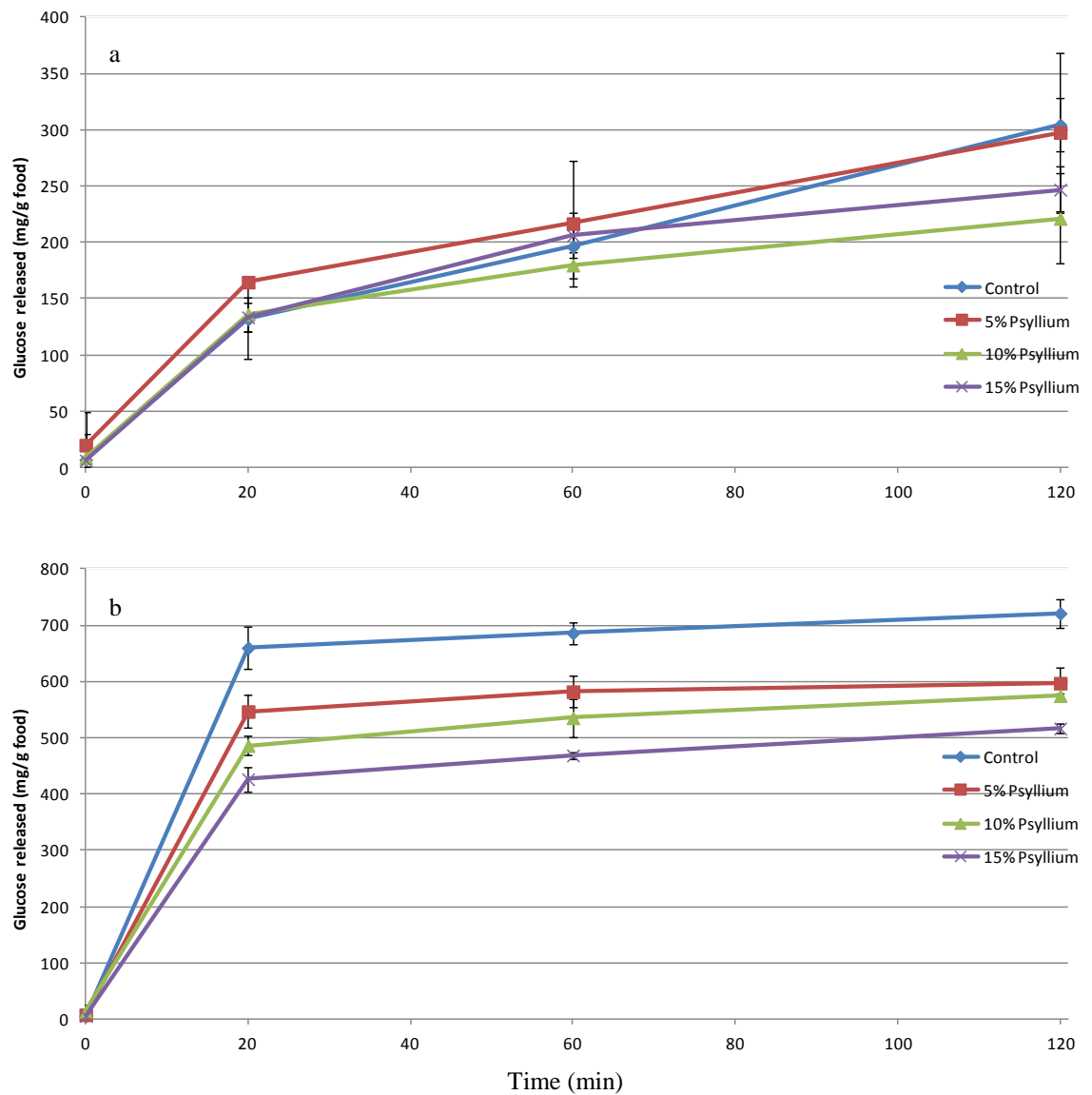


Figure 6.25. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % psyllium (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.26 a and b shows the glucose release for samples containing mushroom waste at 5 %, 10 % and 15 % compared with the control were similar to the results observed previously; the raw samples showed little or no difference between the samples, whereas samples after extrusion exhibited significantly less ($P \leq 0.05$) glucose released at 20 min, 60 min and 120 min compared with the control. Again this ingredient behaved in a dose responsive manner.

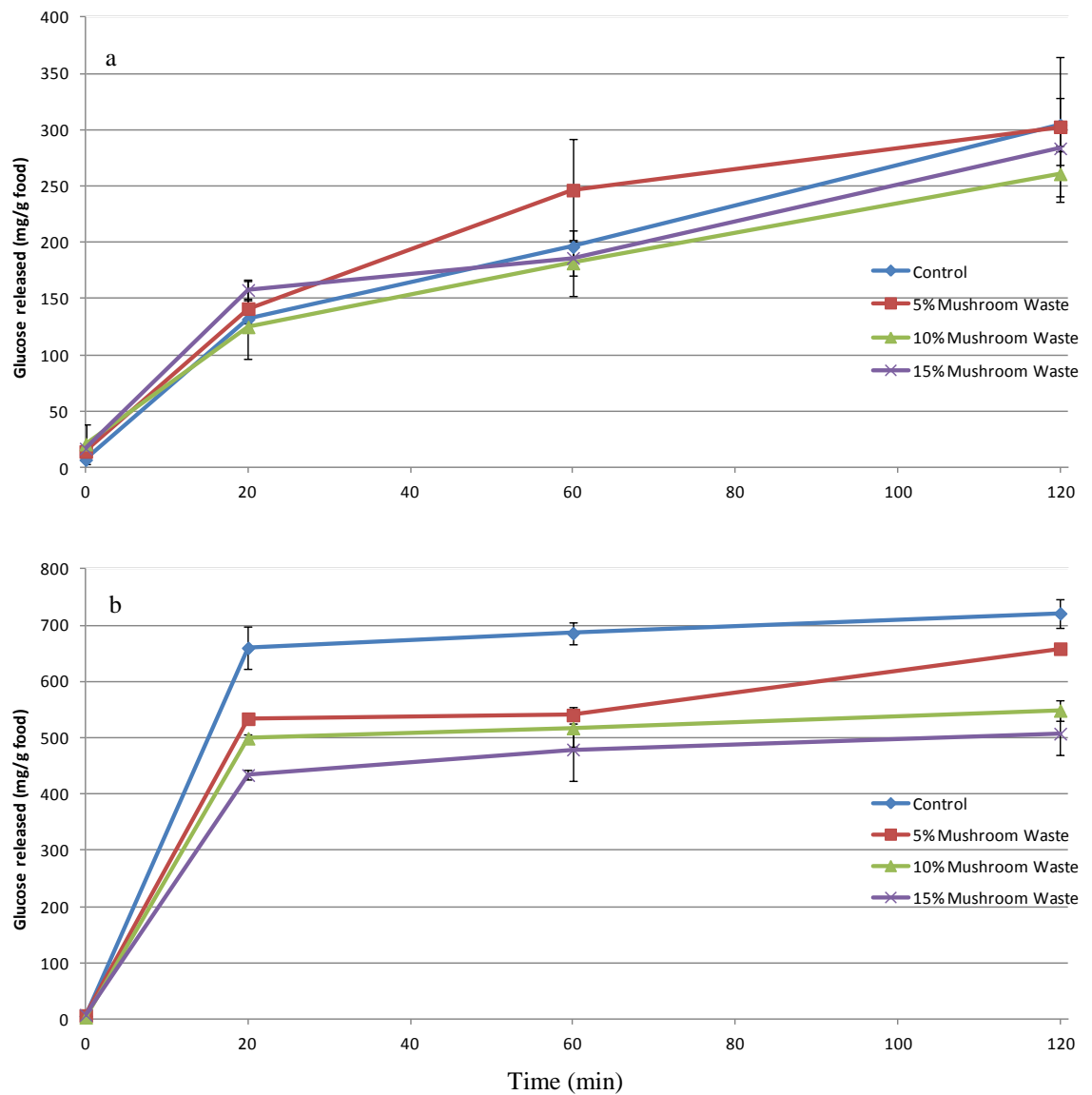


Figure 6.26. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % mushroom waste (n=2) (data expressed as mean values \pm standard deviation).

Similarly, inulin inclusion at 5 %, 10 % and 15 % had no significant effect on the glucose released in the raw sample however after extrusion the amount of glucose is significantly decreased ($P \leq 0.05$) compared to the control, at 20 min, 60 min and 120 min (**Figure 6.27** a and b). There was no clear dose response observed using this ingredient.

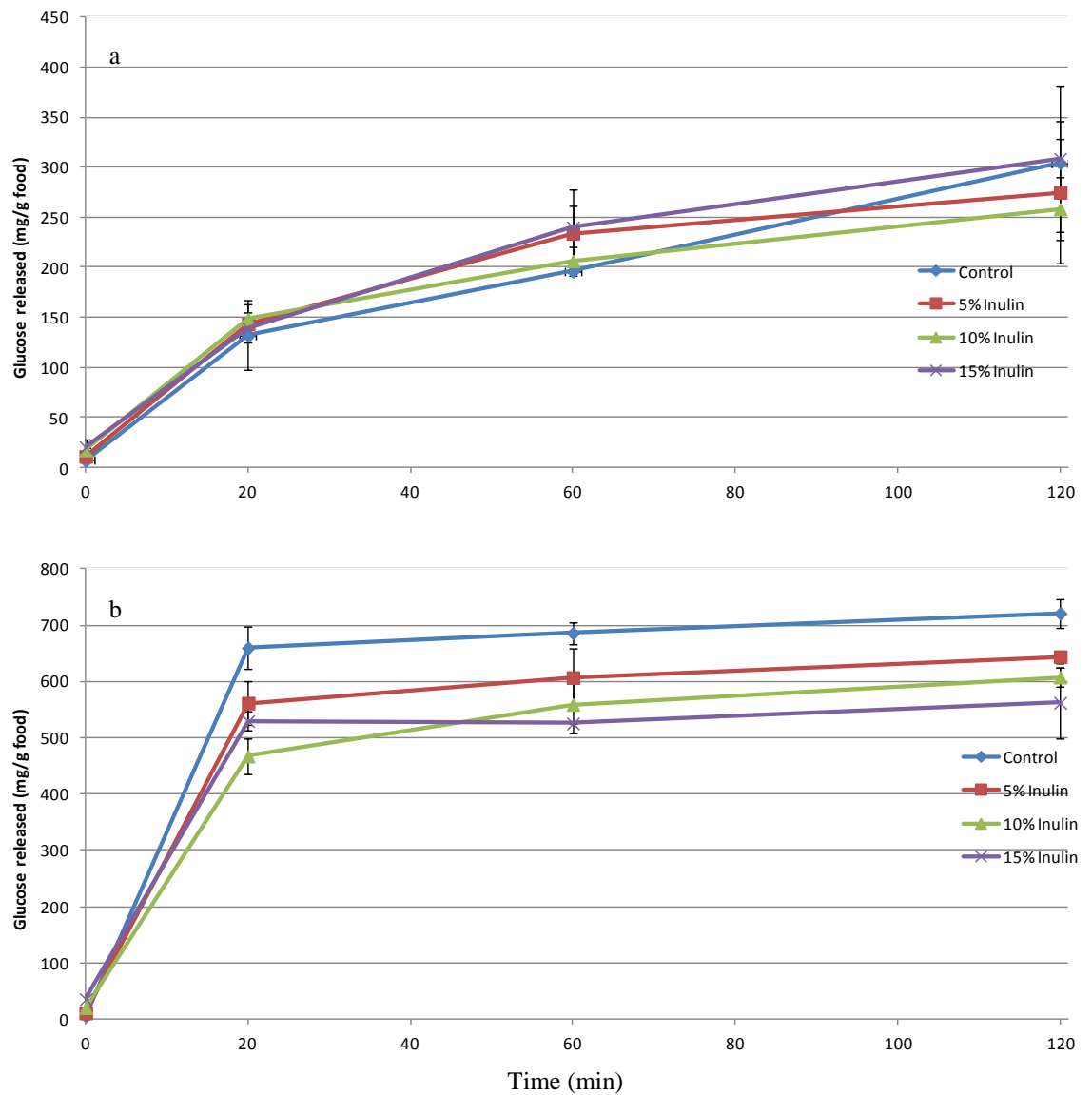


Figure 6.27. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % inulin ($n=2$) (data expressed as mean values \pm standard deviation).

In vitro digestion of the raw samples containing gatifolia showed no significant difference in glucose release compared to the control. Indeed at the 5 % level there was a non-significant increase in glucose release (**Figure 6.28a**). However after extrusion samples containing gatifolia at 5 %, 10 % and 15 % produced significantly less ($P \leq 0.05$) glucose at 20 min, 60 min and 120 min compared with the control (**Figure 6.28b**).

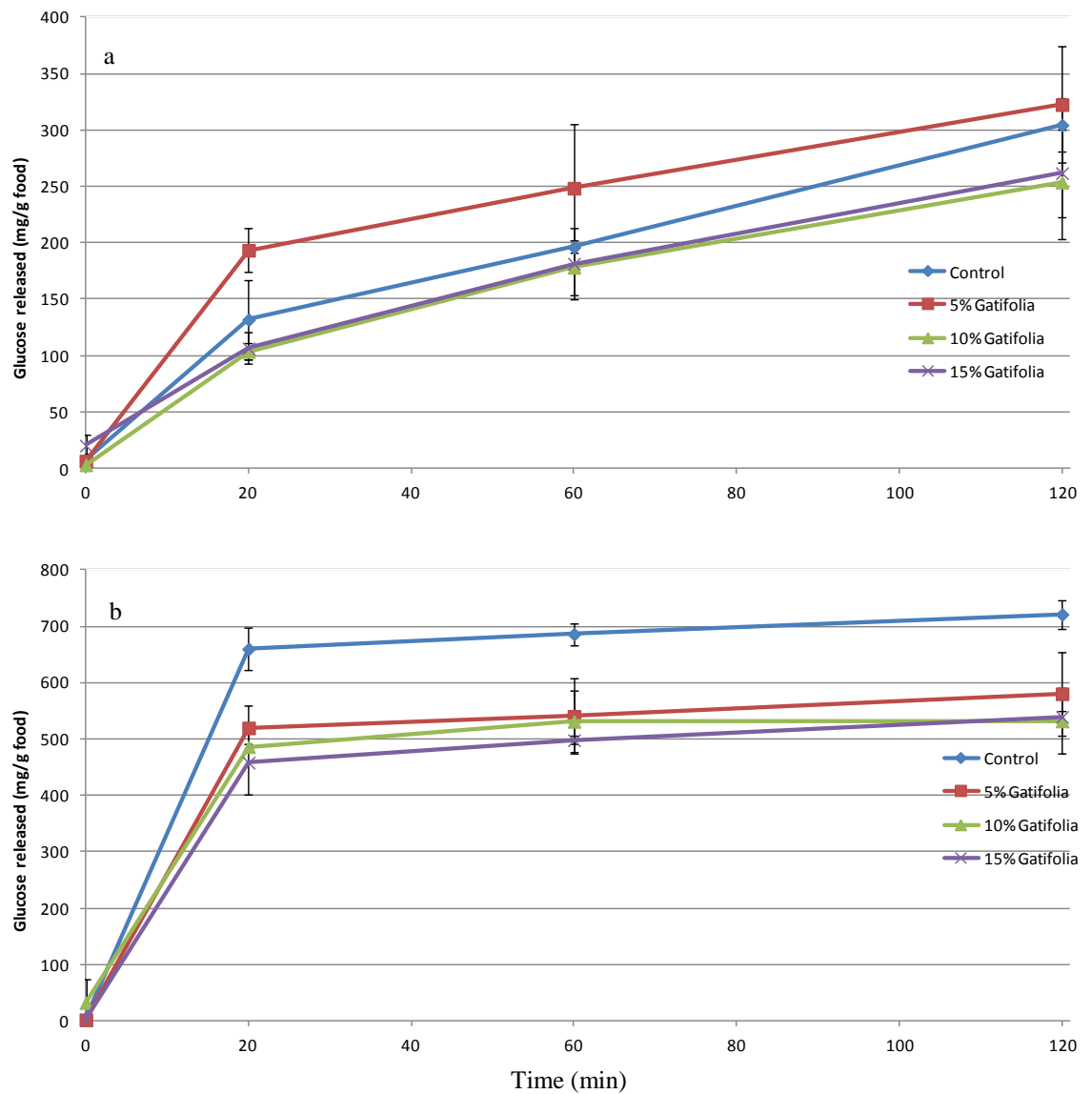


Figure 6.28. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % gatifolia (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.29 a and b shows *in vitro* digestion, glucose released for samples containing super gum at 5 %, 10 % and 15 % compared with the control. Again, in the raw sample there is no significant difference between the samples, however after extrusion all the samples containing super gum have significantly reduced ($P \leq 0.05$) glucose release.

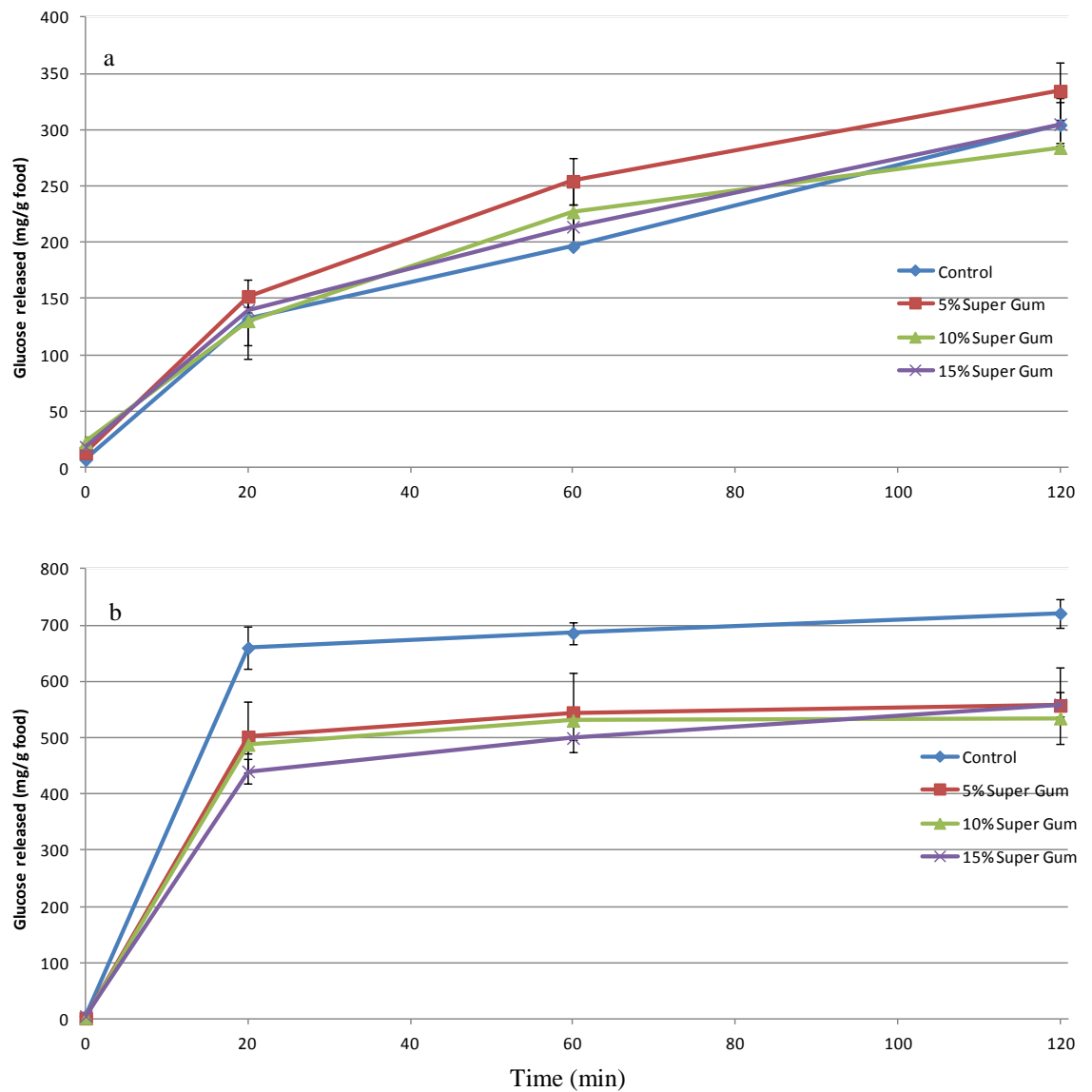


Figure 6.29. *In vitro* glucose release for a) raw b) extruded product, control compared with 5 %, 10 %, and 15 % super gum (n=2) (data expressed as mean values \pm standard deviation).

A product using a high proportion of barley flour was also tested to examine the potential of barley flour as an ingredient in snack foods. It is clear from the graphs that glucose release was not affected at the raw samples whereas after extrusion the glucose release was significantly ($P \leq 0.05$) reduced at 20 min, 60 min and 120 min (**Figure 6.30**).

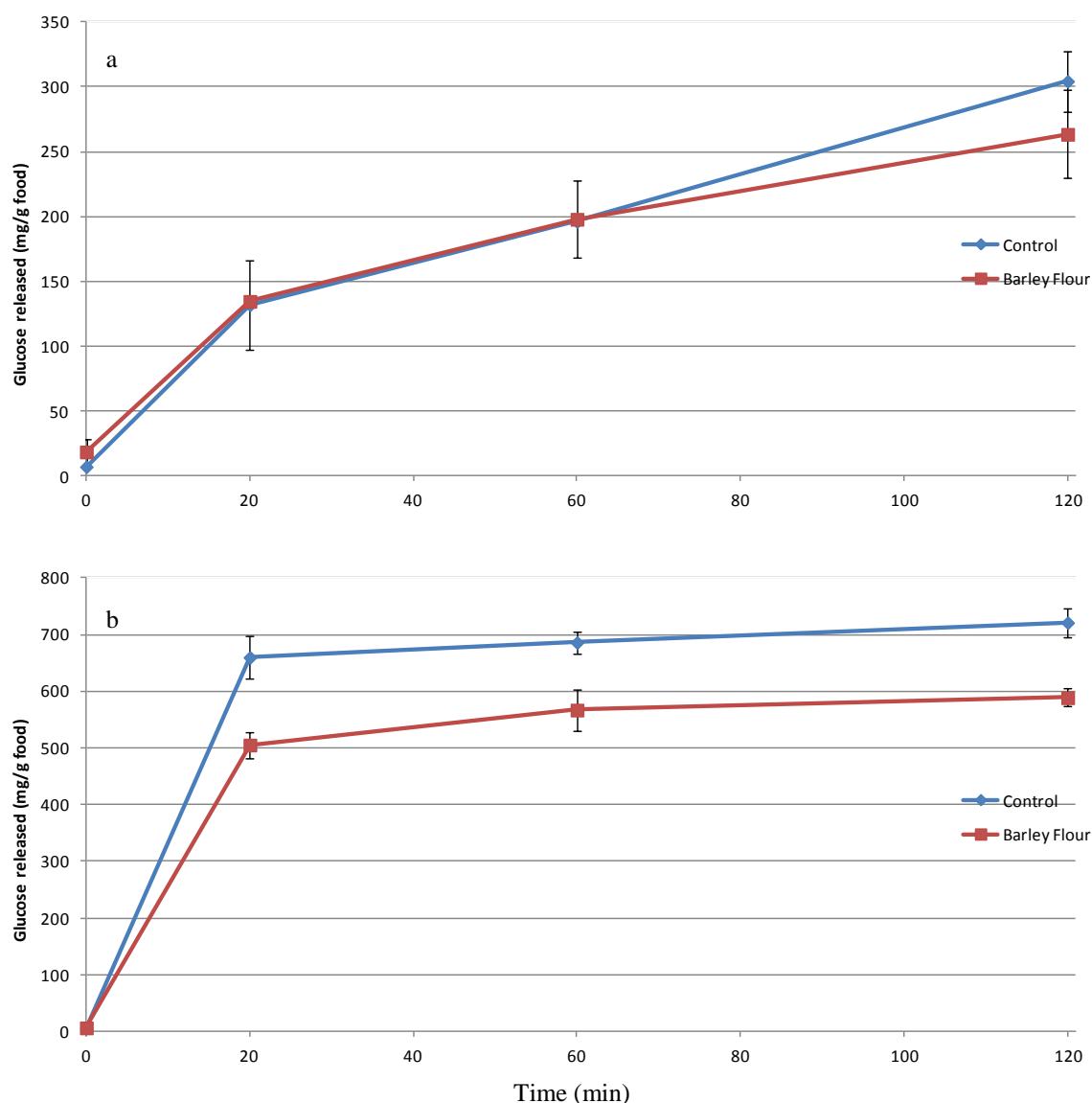


Figure 6.30. *In vitro* glucose release for a) raw b) extruded product, control compared against the barley flour sample (n=2) (data expressed as mean values \pm standard deviation).

For comparative purposes **Figure 6.32** a,b,c and **Figure 6.31** a,b c illustrate the different digestion profiles (and glucose release) of samples at 5 % (a), 10 % (b) and 15 % (c) inclusion levels in both raw and extruded samples. **Figure 6.32** a,b,c clearly illustrates the observation made in the previous figures that the *in vitro* digestion process did not show any significant differences in glucose release over time. The actual values obtained were similar at all inclusion rates and the graphs showed no degree of plateauing. This in itself is interesting as it indicates that starch hydrolysis is not being hampered by ingredient content *per se*. However the overall glucose responses are between 40-60 % of the glucose values observed in the extruded samples.

Extrusion therefore plays a significant role in increasing the availability of starch for hydrolysis and hence the release of glucose during digestion (**Figure 6.32** a, b and c). The pattern of the graphs is also different in that a plateau of glucose release is observed, with high levels of glucose being released during the time point 0-20 min. This confirms that extrusion has a significant role in increasing the level of readily digestible starch. The graphs also indicated that different ingredients (fibres) regulated starch digestion in different degrees. There appears to be a clear dose related effect in terms of increasing fibre inclusions results in a reduction of starch digestibility and glucose release. This could be simplistically due to a reduction in terms of starch concentration, however the reduction of glucose released in the samples containing 15 % psyllium and gatifolia (for example) is greater than the 15 % reduction one would expect from a straight substitution effect.

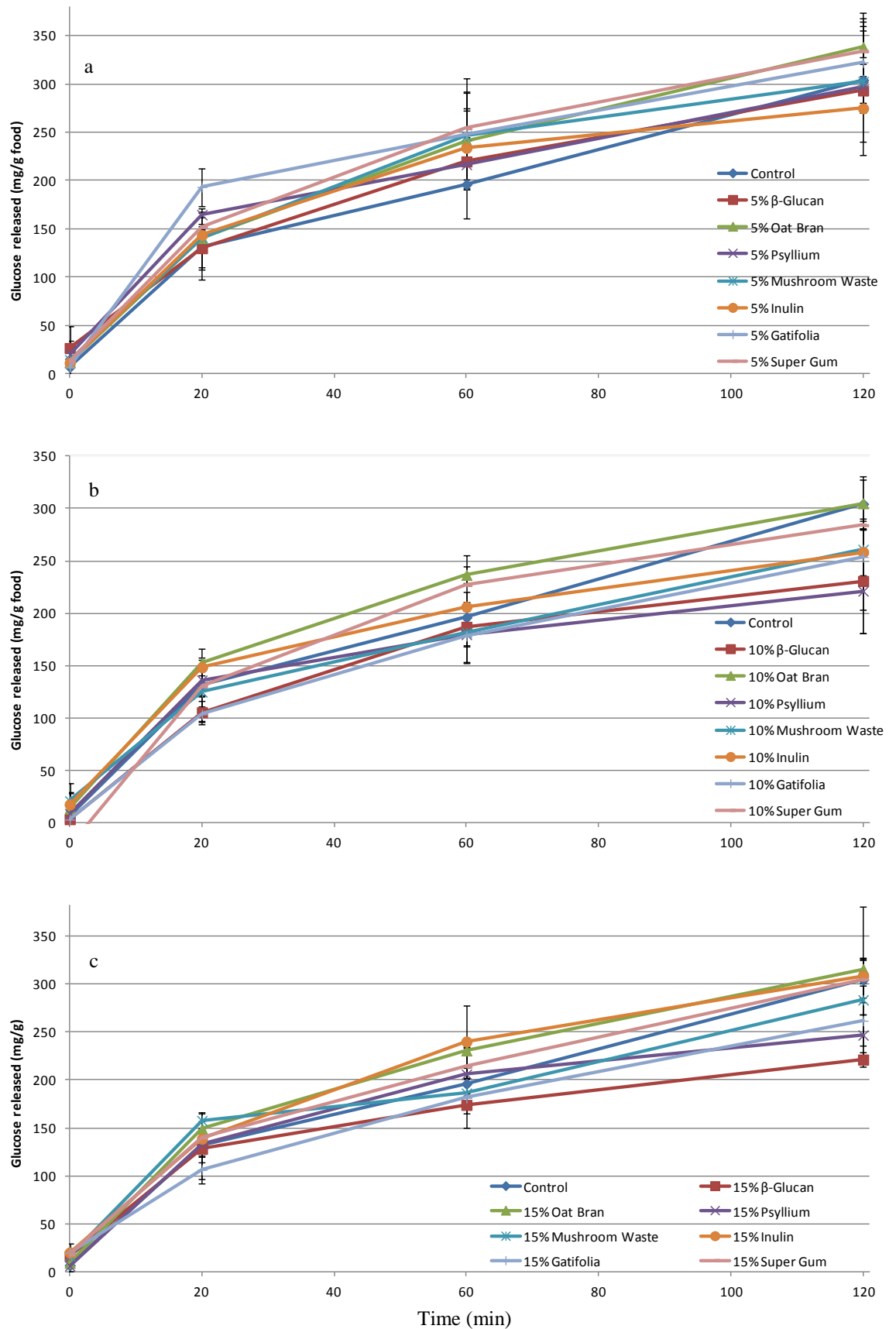


Figure 6.31. *In vitro* glucose release profile of raw mixes for samples containing a) 5 %, b) 10 % and c) 15 % inclusion levels of fibre against a control (n=2) (data expressed as mean values \pm standard deviation).

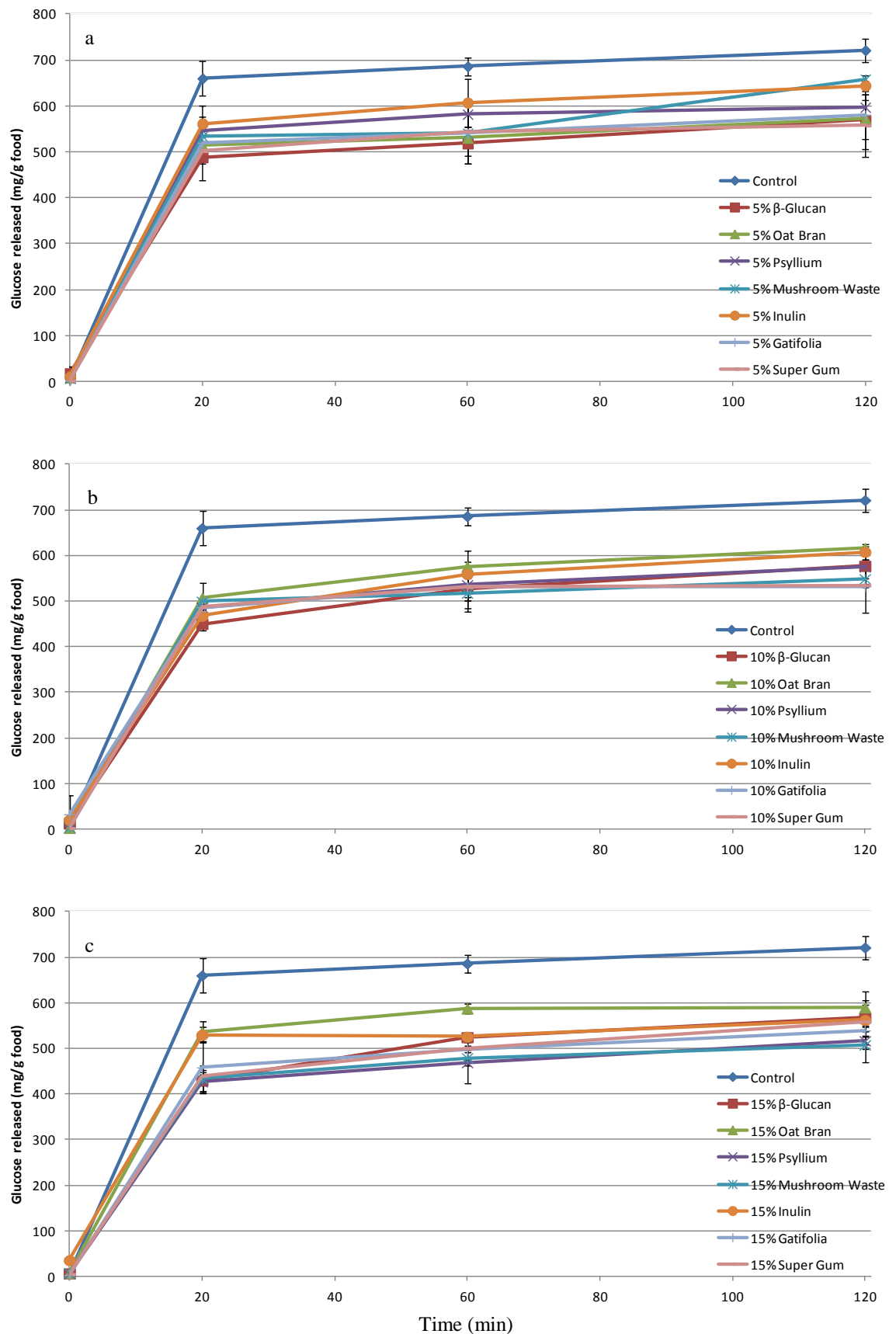


Figure 6.32. *In vitro* glucose release profile of extruded snack products containing a) 5 %, b) 10 % and c) 15 % inclusion levels of fibre against a control (n=2) (data expressed as mean values \pm standard deviation).

The rate of starch digestion (and hence release of glucose) can also be represented incrementally.

Figure 6.33 illustrates such a presentation of the results. Glucose present at the start of the experiment (0 min), during the early stages of starch digestion (0 – 20 min), the mid stages of starch digestion (20-60 min) and the latter part of digestion (60 – 120 min) can be compared relatively easily. Generally the starch fraction of foods is divided into a rapidly digestible fraction (0 – 20 min of the digestion process) and a slowly digestible fraction (20 – 120 min).

Figure 6.33 demonstrates that the control extruded product has a high content of readily digestible starch. In contrast the inclusion of fibre components significantly reduces the extent of readily digestible starch in the sample ($P \leq 0.05$). For instance psyllium and mushroom waste samples exhibited a dose response to reduction of readily digestible starch components; increasing levels of inclusion result in a related reduction of glucose release. Although β -glucan, oat bran, inulin, gatifolia and super gum also show a significant reduction in glucose release compared to the control sample, this reduction did not appear to be dose related ($P \leq 0.05$).

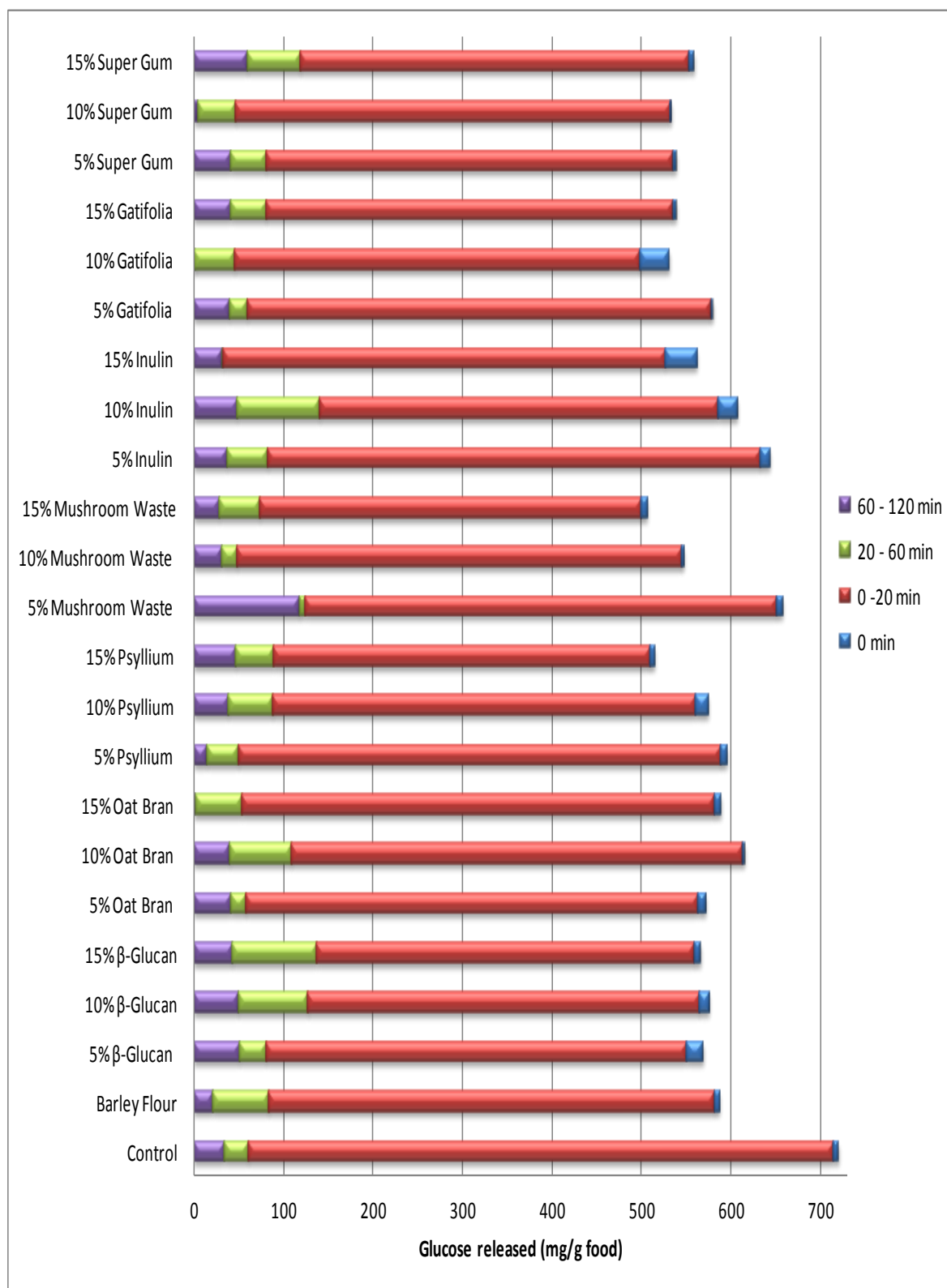


Figure 6.33. *In vitro* incremental glucose release profile of extruded snack products (n=2) (data expressed as mean values \pm standard deviation).

Figure 6.34 illustrates the standardised area under the curve measurements of glucose released from the extruded snack products resulting from the *in vitro* digestion protocol. All samples which had fibre inclusions showed a significantly reduced glucose AUC compared to the control sample ($P \leq 0.05$). In some samples this followed a dose response (for instance psyllium, mushroom waste and gatifolia inclusion), however others showed either no clear dose response or a negative correlation. The results from oat bran inclusion were of interest in that increasing the inclusion rate of oat bran resulted in slight increases in glucose AUC (although the glucose AUC of the 15 % inclusion level of oat bran was still significantly lower than that of the control sample).

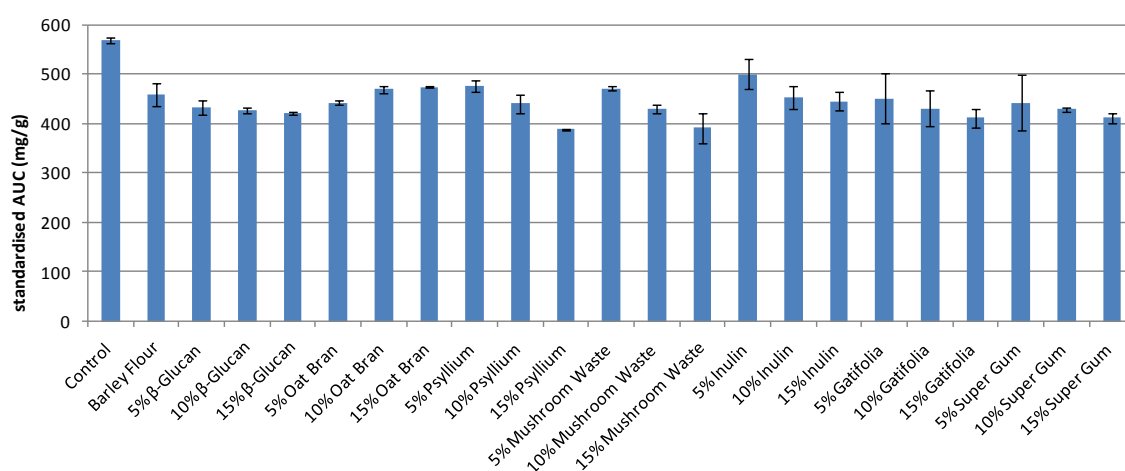


Figure 6.34. Standardised area under the curve glucose release measurements obtained from *in vitro* procedures of extruded snack products (n=2) (data expressed as mean values \pm standard deviation).

Chapter 7

Product Characteristics of Phase Two Extruded Snack Products and the Results of Their Inclusion in an Intervention Trial.

7.1 Introduction

Following the preliminary screening of the different fibre samples and the different inclusion levels, 2 fibre products were selected to use in an intervention trial. The trial was designed to investigate the *in vivo* effects of fibre inclusion in snack food products in order to establish a relationship between fibre inclusion and glucose manipulation. Two fibres used in the initial extrusion process stood out amongst the other fibre components. Psyllium and oat bran both contributed the greatest increase in fibre levels when incorporated into the extruded snack products as illustrated in **Figure 6.5**. In addition, psyllium fibre showed the greatest reduction of glucose AUC in the *in vitro* starch digestion protocol, as observed in **Figure 6.34**. The inclusion of oat bran was made on the basis of extensive research publications linking the ingestion of oat bran to both cholesterol and postprandial glucose manipulation. In addition, **Figure 6.34** illustrated an interesting relationship in terms of the level of oat bran inclusion affecting glucose AUC values. Inclusion at 15 % was chosen in order to try to achieve the maximum amount of fibre inclusion in a product as possible. Based on calculations from **Figure 6.5**, a 15 % inclusion of oat bran or psyllium had the potential of generating snack products with up to 20 % fibre.

7.2 Density and expansion properties of phase two snack products.

Extruded snack products were produced using the experimental protocol identified in **section 5.2**. The product density and expansion ratio of the control snack product and snack products with 15 % psyllium and oat bran were determined **Table 7.1**. Although product density of the samples containing 15 % fibre inclusion were statistically higher than that of the control sample, the expansion ratio of the control product and the product containing 15 % psyllium were not statistically different. This meant that the processing parameters had been manipulated to try to provide a product with as close a similarity to the control sample as possible.

Table 7.1. Product density and expansion ratio for phase two extruded snack products (data expressed as mean values \pm standard deviation).

	Product density (kg/m ³)	Expansion ratio (%)
Control	129.64 \pm 1.51	259.58 \pm 12.88
Oat Bran	154.08 \pm 1.00	212.70 \pm 18.30
Psyllium	145.11 \pm 5.86	239.80 \pm 20.03

7.3 Water absorption index of phase two extruded snack products

Figure 7.1 illustrates the water absorption index values for the extruded control product as well as the extruded products with 15 % inclusion of psyllium and oat bran. No significant difference ($P \leq 0.05$) was observed between the control and the product with 15 % inclusion of oat bran. However the inclusion of 15 % of psyllium increased the WAI of the product compared to the control and oat bran samples. This is likely to be an effect of the water hydration properties of the psyllium material as previously observed.

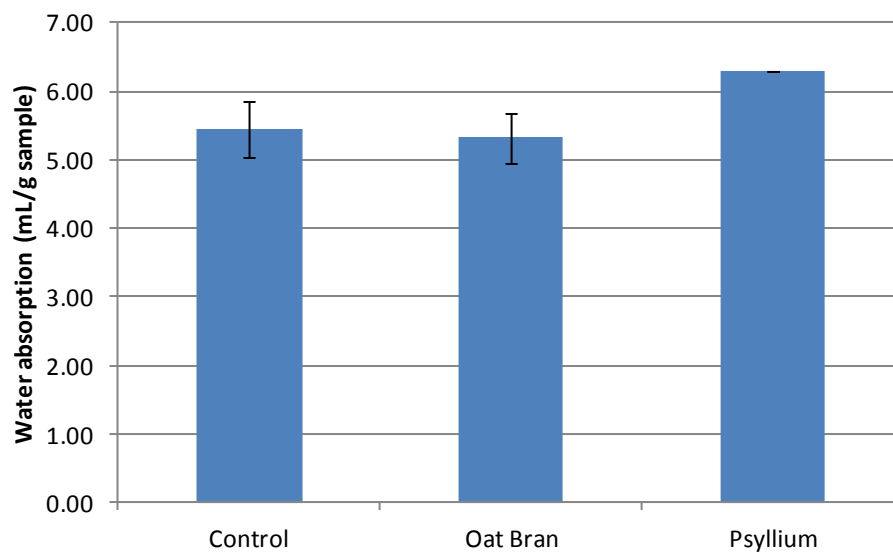


Figure 7.1. Water absorption index of extruded snack products (n=2) (data expressed as mean values \pm standard deviation).

7.4 Water solubility index of phase two extruded snack products

The water solubility of the extruded snack products to be used in the *in vivo* clinical trial is shown in **Figure 7.2**. No significant difference ($P \leq 0.05$) was observed between the control and the oat bran product, whereas the water solubility index of the psyllium enriched products were significantly reduced compared to both the control and oat bran samples.

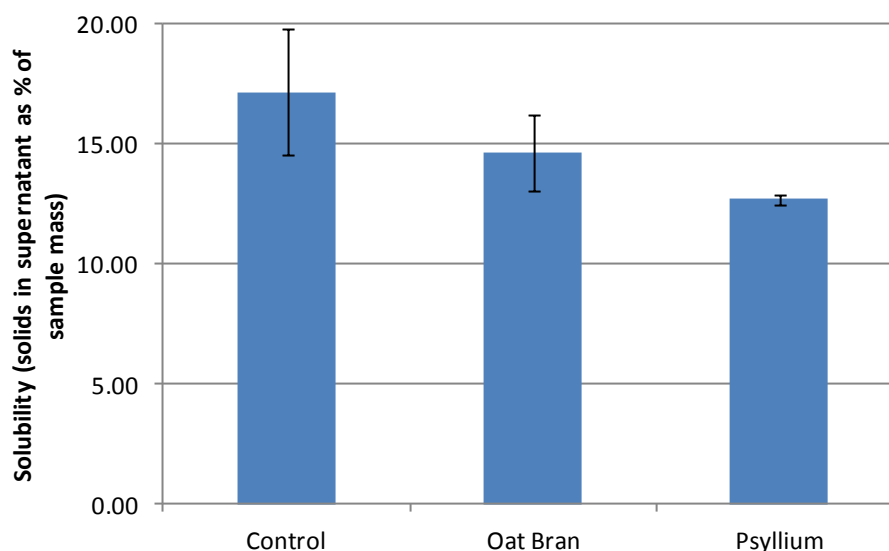


Figure 7.2. Water solubility index of extruded snack products used in the *in vivo* clinical trial (n=2) (data expressed as mean values \pm standard deviation).

7.5 Chemical composition of phase two extruded snack products

The chemical composition of the extruded samples were determined (shown in **Table 7.2**) in order to calculate the mass of sample required to deliver 25 g of carbohydrate to individuals participating in the *in vivo* trial. The protein contents of all samples were similar. The fibre contents of the oat bran and psyllium samples were confirmed at 20.10 % and 19.80 % respectively. This was a significant ($P \leq 0.05$) increase in terms of the control sample and was consistent with the aim of obtaining a fibre rich product containing approximately 20 % fibre. As a result of the differences in fibre content, the digestible carbohydrate fraction of the oat bran and psyllium products were significantly lower than the control sample. In order to achieve a product loading of 25 g of available carbohydrate per participant the mass of sample to be given to the participants was calculated as 30.6 g , 38.58 g, and 37.65 g for the control sample and products with oat bran and psyllium

inclusion respectively. This corresponded to a fibre loading of 1.8 g, 7.7 g and 7.6 g for each portion of the control product and the products containing oat bran and psyllium respectively. Such a loading represents approximately 40 % of the RDI (recommended daily intake) of fibre (NHS 2011) being met by ingesting an approximately 38 g portion of the fibre rich products.

Table 7.2. Chemical composition (g/100g as is basis) of phase two extruded snack products

	Ash	Protein	Moisture	Fibre	Fat	Carbohydrate	Mass of sample to give 25g COH
Control	1.00	8.70	2.00	5.90	0.70	81.70	30.60
Oat Bran	1.30	8.80	3.50	20.10	1.50	64.80	38.58
Psyllium	1.50	8.50	3.00	19.80	0.80	66.40	37.65

7.6 Rapid Visco Analyser pasting profiles of phase two extruded products

Table 7.3 illustrates the pasting properties (peak viscosity and final viscosity) of the extruded samples produced for the glucose *in vivo* trial. The inclusion of oat bran and psyllium at 15 % level significantly ($P \leq 0.05$) increased the peak viscosity of the extruded snack products. Psyllium fibre inclusion increased the peak viscosity values of the product compared to the oat bran inclusion, indicating the viscosity altering properties of psyllium fibre. Although no significant difference was observed between the oat bran and control samples in terms of final viscosity, inclusion of psyllium fibre significantly increased the final viscosity of the extruded snack samples.

Table 7.3. Pasting profiles of extruded snack products to be used in the *in vivo* trial (data expressed as mean values \pm standard deviation).

	Peak viscosity (cP)	Final viscosity (cP)
Control	264.50 \pm 12.02	198.00 \pm 4.24
Oat Bran	307.50 \pm 9.19	205.00 \pm 4.24
Psyllium	616.00 \pm 9.90	584.00 \pm 5.66

7.7 Texture attributes of phase two extruded snack products.

The texture (peak force and number of peaks) of the extruded samples were evaluated in order to determine the similarities and differences of the products. **Table 7.4** illustrates that the peak force required to compress the extruded snack products were not significantly ($P \leq 0.05$) different. However there is a non-significant trend of fibre inclusion increasing the peak force of the extruded products. No significant difference was observed between the control sample and the extruded product with psyllium inclusion for the number of peaks observed, but the oat bran enriched product showed a reduced number of peaks compared to the control and psyllium products. These observations indicate that the psyllium fibre extruded product was generally similar to the control in terms of textural attributes whereas the oat bran product was slightly different in terms of potential crispiness of the product.

Table 7.4. Textural attributes of extruded samples used for the *in vivo* intervention trial (data expressed as mean values \pm standard deviation).

	Peak force (kg)	Number of peaks
Control	3.37 \pm 0.43	38.57 \pm 3.82
Oat Bran	3.87 \pm 0.62	28.20 \pm 3.05
Psyllium	4.29 \pm 0.34	39.80 \pm 2.62

7.8 *In vitro* analysis of phase two extruded snack

In vitro starch digestibility determinations were conducted on the extruded snack products to be used for the *in vivo* intervention trial. The amount of glucose released in the samples is shown in **Figure 7.3**. Both the oat bran and psyllium samples exhibited a significantly ($P \leq 0.05$) reduced glucose response over the first 20 min of the digestion period. There-on-in no significant difference was observed between the control and oat bran samples. Interestingly the greatest response was observed with the inclusion of psyllium fibre.

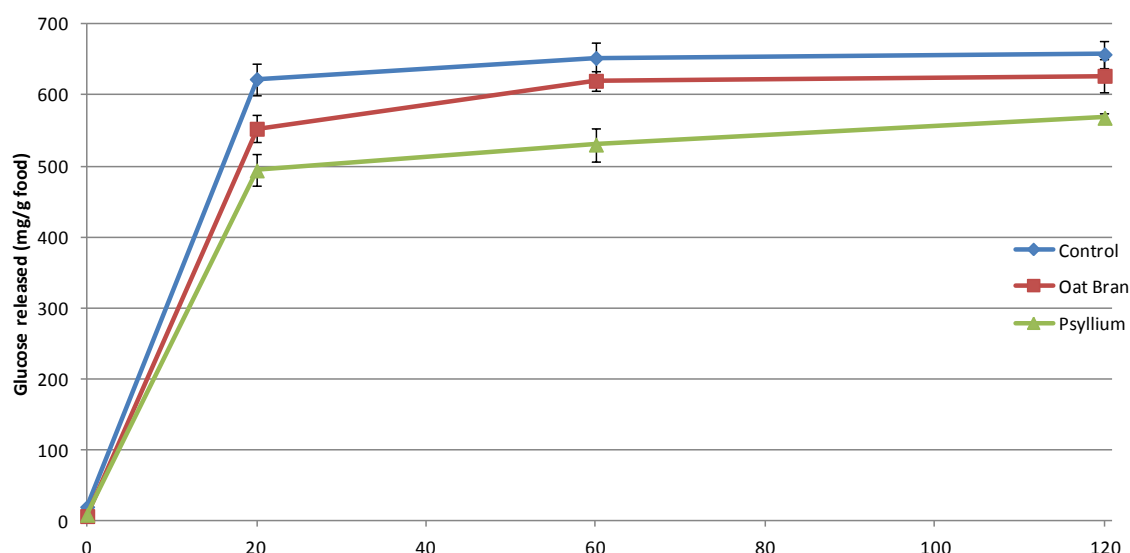


Figure 7.3. *In vitro* analysis of extruded snack products to be used in the *in vivo* intervention trial (n=2) (data expressed as mean values \pm standard deviation).

The incremental glucose release over the 120 min digestion process is illustrated in **Figure 7.4**. Both oat bran and psyllium inclusion significantly ($P \leq 0.05$) reduced the glucose release over the initial 20 min. Both oat bran and psyllium inclusion showed a significant reduction in rapidly digestible carbohydrate (glucose released during 0-20 min). Oat bran and psyllium fibre inclusion were significantly different from the control sample in terms of total incremental glucose release.

The incremental area under the curve glucose response is demonstrated in **Figure 7.5**. The overall glucose AUC of both oat bran and psyllium samples was significantly ($P \leq 0.05$) reduced compared to that of the control snack product. The AUC for the psyllium snack product was also significantly lower than that of the oat bran extruded snack product. Such an observation indicates that the inclusion of psyllium into extruded snack products could have a larger contribution in the reduction of *in vivo* blood glucose response than that of oat bran inclusion into snack products.

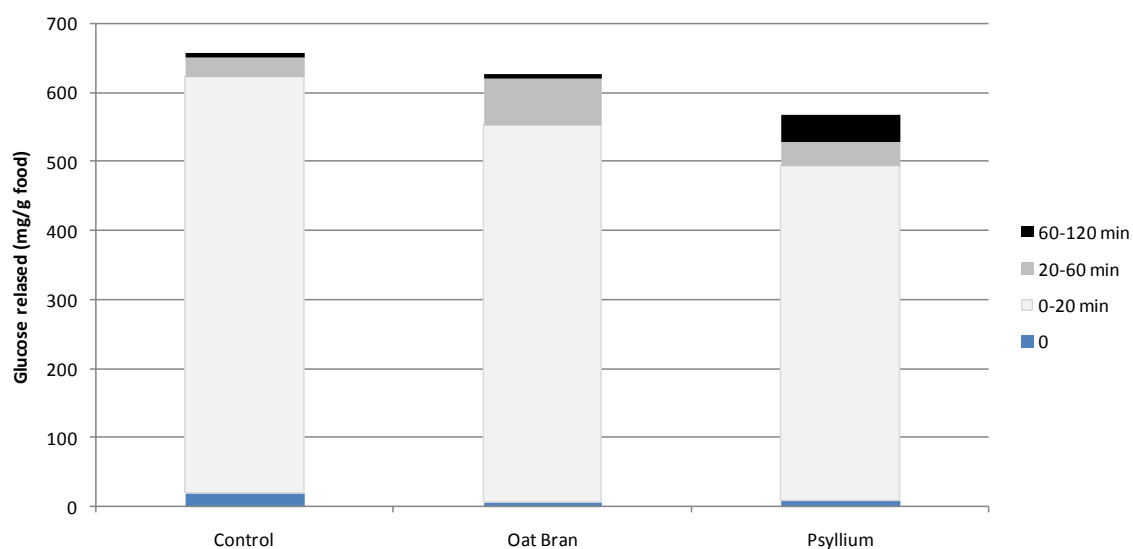


Figure 7.4. Incremental glucose release using *in vitro* analysis of extruded snack products to be used in the *in vivo* intervention trial (n=2).

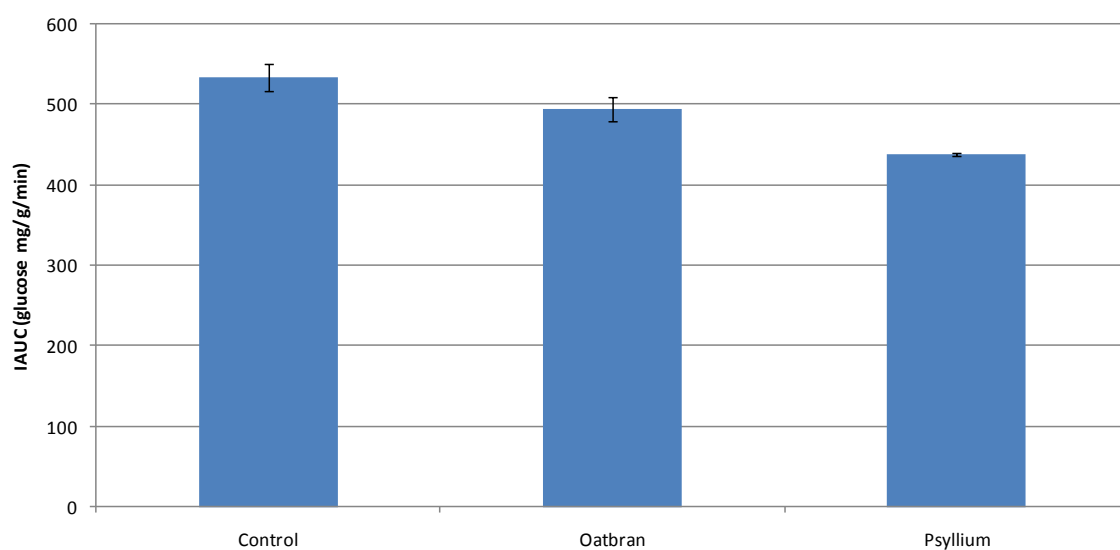


Figure 7.5. Incremental glucose AUC using *in vitro* analysis of extruded snack products to be used in the *in vivo* intervention trial (n=2) (data expressed as mean values \pm standard deviation).

7.9 *In vivo* postprandial glucose response to ingestion of phase two snack products

The postprandial blood glucose responses to the three snack products were evaluated in a clinical trial involving 12 healthy volunteers. All participants were requested to ingest a portion of snack products with 25 g loading of digestible carbohydrate. In addition a drink containing 25 g glucose was also used (as per previous research conducted by Kendall *et al.*, (2008)).

Figure 7.6 shows the difference in blood glucose levels from the fasting baseline for all treatments. Each figure compares the response of individuals after consuming the control extruded sample containing 25 g of available carbohydrate against the different treatments as recorded over 120 min. The psyllium snack had a much flatter blood glucose response compared to the oat or control snacks or the drink. Interestingly the oat bran snack elicited a greater blood glucose response than the control however it did not return to baseline at 120 min unlike the control which returned to baseline before 100 min.

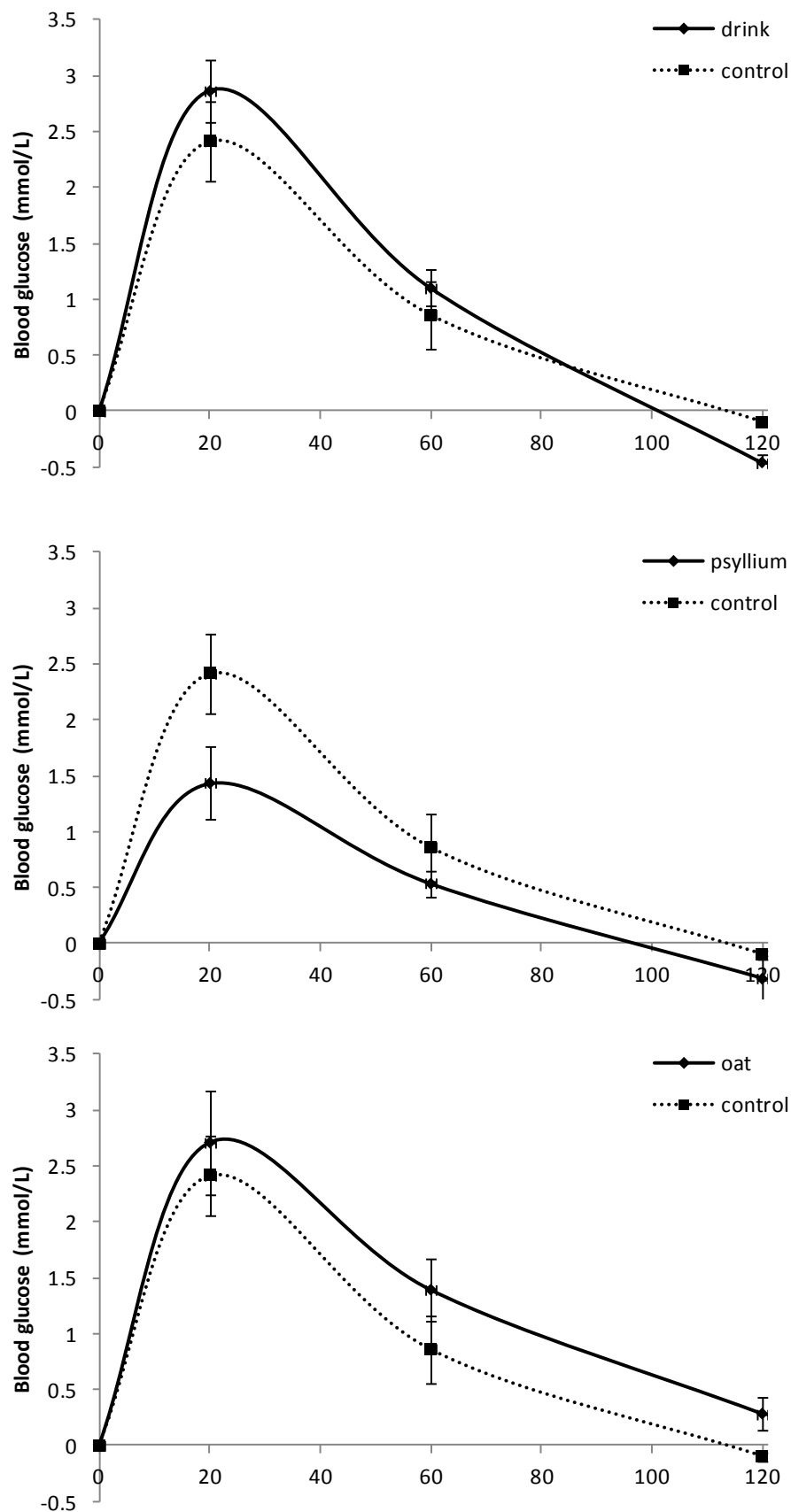


Figure 7.6. Postprandial blood glucose response following ingestion of control drink, control snack product, oat bran enriched snack product and a psyllium enriched snack product (n=12) (data expressed as mean values \pm SEM).

The standardised AUC values following ingestion of the trial samples are shown in **Figure 7.7**. The standardised AUC for the psyllium containing samples was significantly ($P \leq 0.05$) less than those recorded for all other samples. Indeed there was no significant difference between the oat bran snack and glucose drink samples. Both of these samples exhibited significantly increased AUC compared to the control extruded snack product.

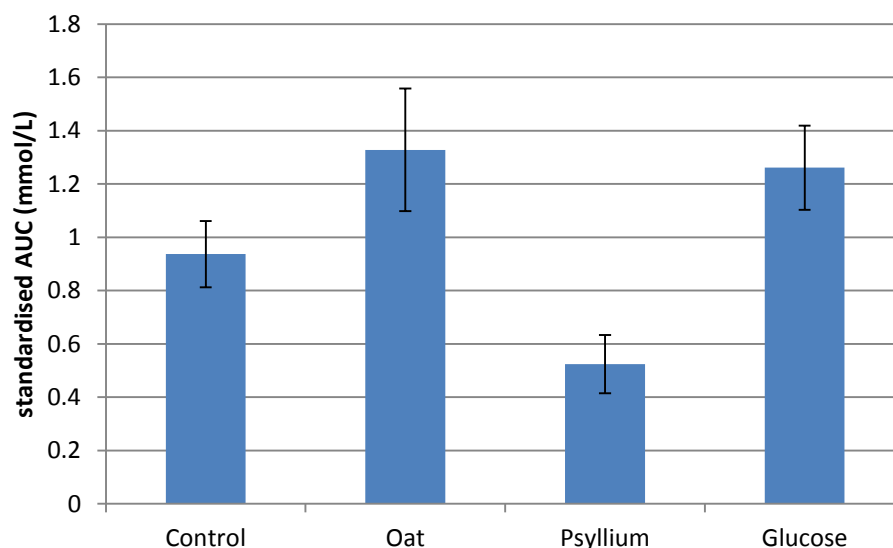


Figure 7.7. Postprandial blood glucose AUC response following ingestion of control drink, control snack product, oat bran enriched snack product and a psyllium enriched snack product (n=12) (data expressed as mean values \pm standard deviation).

7.10 Sensory analysis of extruded snack products

Alongside the *in vivo* postprandial glucose response trial a non-trained consumer sensory analysis trial was conducted on the three extruded samples. The aim of the sensory trial was to determine the effect of oat bran and psyllium inclusion on the consumer appreciation of the snack products in comparison to the control sample. In total 50 volunteers participated in the evaluation of which 54 % were female and 46 % male. Of the volunteers 80 % of them stated that they regularly ate snack products.

Figure 7.8 demonstrates the sensory characteristics assessed by the sensory panel. No significant ($P \leq 0.05$) difference was observed between any of the extruded snack products and their sensory parameters. The closeness of the fibre rich products to the control sample is indicated in **Figure 7.8** and represents the successful formulation and production of the extruded snack samples.

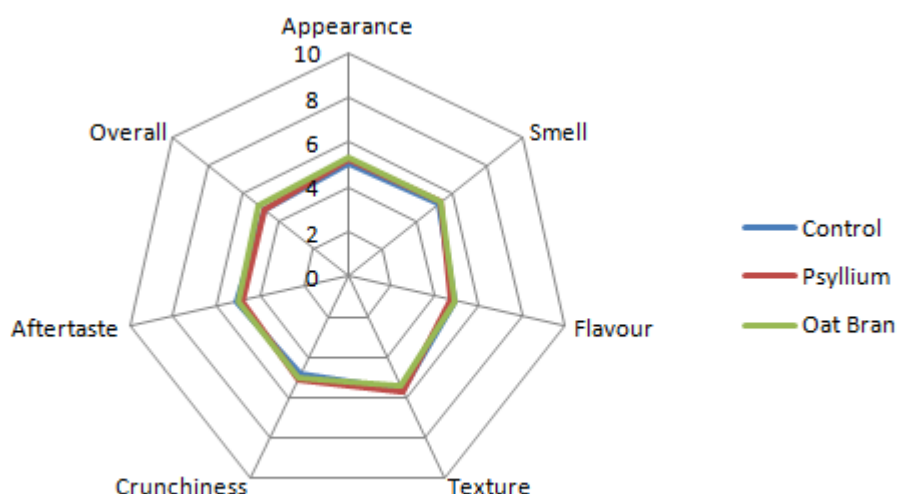


Figure 7.8. Sensory characteristics of the control, oat bran and psyllium extruded snack products as perceived by a non-trained consumer taste panel (n = 50) (data expressed as mean values).

Examining the individual parameters the values for appearance ranged were 5.04, 5.24 and 5.29 for the control, oat bran and psyllium samples respectively. The 10 cm linear evaluation of acceptability ranged from 0 (not acceptable), 5 (neither acceptable or unacceptable) and 10 (acceptable). The aroma characteristics also ranked close to mid point (ranging from 5.26 - control samples, 5.32 – psyllium samples, 5.37 – oat bran sample). Consumers ranked flavour as 4.91 – control, 4.76 – psyllium product and 4.88 – oat bran product. The texture of the products showed a trend to greater acceptability amongst samples with values varying between 5.59 – control, 5.74 – psyllium products, 5.44 – oat bran. Crunchiness of the products showed a trend to slightly less acceptability amongst samples with values varying between 4.86 – control, 5.07 – psyllium products, 5.00 – oat bran. The psyllium and oat bran samples (4.86 and 4.99 respectively scored slightly less favourably in aftertaste than the control sample (5.06). This point was also picked up in consumer comments regarding blandness of the product and aftertaste (appendix, ‘collection of sensory responses’).

Overall acceptability showed no significant difference between the three products. This illustrates that it is possible to produce a high fibre product using oat bran and psyllium that is similar in product characteristics to a control (low fibre) product. From a food processing view point this opens up a tremendous opportunity to be able to manufacture high fibre snack products with good consumer acceptability characteristics.

Chapter 8

Discussion

8.1 Introduction

The primary focus of this research has been the utilisation of different dietary fibres in designing snack products which can mediate the postprandial glycaemic response of individuals. Recent trends have shown an increased consumption of RTE foods in the U.K. and globally. The most recent information from the Euromonitor International report (2011) indicated a 4 % increase, globally, in retail value between 2008 and 2009. In the U.K. sales of extruded snack product have increased by 13 % between 2005 and 2010. When combining these figures with the research showing an increase in snacking frequency during a day (Bertéus-Forslund *et al.*, 2005; Johnson & Anderson, 2010) it can be suggested that snacking and weight gain go hand in hand. Considering the fact that the majority of snack products are energy dense it becomes important to try to manipulate their nutritional value while also maintaining consumer acceptability. The results presented in this thesis significantly contribute to our understanding of how both insoluble and soluble fibre components can be successfully included into products which are potentially useful in the influence of dietary glycaemic responses. This chapter reviews the results obtained during the overall research platform and evaluates aspects in relation to previous published results.

8.2 Effect of DF on the physicochemical properties of snack foods

In terms of consumer acceptability the physical nature of an extruded snack product is of great importance. Without good texture, a suitable crispy/crunchy balance and an acceptable appearance, new products will not succeed (Kim *et al.*, 2009). Two accepted industrial measurements related to consumer acceptability are product expansion and density. Consumer preference is generally for a highly expanded and low density product in order to impart desired crispy/crunchiness from a snack product (Lue *et al.*, 1991; Guy, 2001b). The expansion results shown in **Figure 6.15** show that there is a high degree of variability indicating that with careful control in the manufacturing process high fibre samples could be produced with similar expansion characteristics to the control, as illustrated in the production of the samples for the intervention study (**Table 7.1**). Density

(**Figure 6.16** and **Table 7.1**) shows less variability within the samples probably due to the fact that density is measured using a large number of collets for each measurement whereas expansion is measured using individual collets randomly taken from the sample.

In order to attempt to understand the interrelationships between fibre content and the physicochemical quality of foods a Pearson's correlation (Minitab 16) was conducted on all data from the samples manufactured during the first stage of the research (**Table 8.1**).

Table 8.1. Pearson's correlation of snack food composition and physicochemical properties, all fibres.

	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.507	0.195	-0.038	-0.271	0.067	0.137	0.403
Starch		-0.292	-0.391	0.594	-0.253	-0.123	-0.308
Exp			-0.31	-0.025	0.843	0.536	0.51
Den				-0.141	-0.4	-0.264	-0.093
TH					-0.45	0.329	0.126
TP						0.583	0.545
PV							0.8
FV							

Exp- expansion; Den- density; TH- texture hardness value; TP- texture number of peaks value; PV- pasting peak viscosity; FV- pasting final viscosity. Highlighted correlations significant at $p \leq 0.05$, blue representing a negative and red representing a positive correlation ($n = 144$).

When you examine the correlations of the whole range of samples it is clear that fibre has a negative effect on starch content of the snack products. This is a self-evident correlation as simplistically the more fibre as a percentage of product weight you have the less starch you have in that sample as you are replacing starch with fibre. No significant correlations could be observed between fibre levels and any of the physicochemical characteristics of the snack products. Although this suggests that fibre does not affect the physical nature of foods, this is too superficial as the correlations of individual fibre material (**Table 8.2**) later illustrate. Surprisingly the results indicate that there is no correlation between starch and the physicochemical properties of the extruded snack products. This is surprising in that previous literature has suggested positive correlations between starch and expansion ratio, peak viscosity and final viscosity of products and negative correlations between starch and density and hardness of products (Van Hoan *et al.*, 2009; Robin *et al.*, 2011a; Sompong *et al.*, 2011).

Another significant correlation (taking into account all the data acquired from the various fibre materials) is a positive correlation between expansion ratio and the number of peaks recorded during texture analysis of samples. This has been observed by previous researchers using a range of cereal based products in that a more aerated expanded product tends to be brittle in nature creating more fracture events during a compression test (Yao *et al.*, 2006; Dehghan-Shoar *et al.*, 2010). It is interesting to note the positive correlations between expansion ratio and both peak viscosity and final viscosity (as measured by the RVA). Such a correlation does not appear to have been made previously and can provide an insight as to how the chemical components of the snack products affect the physical nature of the product. Conventionally it is assumed that the expansion ratio of extruded snacks is related to the amount of super-heated water leaving the product at the die face (flashing off as steam) and this event has been reported in numerous papers studying extrusion of cereal products (Altan *et al.*, 2008; Alavi, 2011). This has also been related to the degree of “cook” of the product and the amount of starch gelatinisation occurring in the sample (Hagenimana *et al.*, 2006; Mahasukhonthachat *et al.*, 2010; Karkle *et al.*, 2012). The pasting properties of extruded snack products have also been shown to be related to the degree of starch gelatinisation occurring during extrusion, in that the greater the degree of “cook” or starch gelatinisation during extrusion, the lower the peak and final viscosity values of the extruded snack products (Ryu *et al.*, 1993; Gupta *et al.*, 2008; Robin, *et al.*, 2011b). There are three possible hypotheses (H) for the correlations observed in **Table 8.1, 8.2, 8.3 and 8.4.**

H1- the starch is not gelatinising during the extrusion process of these highly expanded products

H2- the fibre components within the snack product are contributing to viscosity of the extruded samples and increasing the peak and final viscosity readings due to their water retention properties

H3- the fibre components more competitively absorb moisture during the mixing stages of the extrusion process than starch granules and that on extrusion the starch granules do not have excess moisture and so show a reduced extent of gelatinisation.

The competitive nature of fibres in absorbing moisture (compared to protein and starch components) is well documented (Holtekjølen *et al.*, 2008; Goldstein *et al.*, 2010) and accounts for the use of many fibres as “gums and stabilisers” for the food industry. **Figure**

6.6 and **Figure 6.7** illustrated the water absorption index values of the fibre ingredients and raw recipe mixes respectively. It could be observed that barley flour, β -glucan, psyllium and mushroom waste all exhibited increased water absorption index compared to the control samples. This observation is in line with previous research suggesting that complex high molecular weight fibres exhibit high water absorption values (Chaplin, 2003; Dikeman & Fahey, 2006).

Table 6.4 Indeed **Table 6.4** illustrates that the pasting properties of these fibre mixes are significantly increased compared to the control. The extent to which this water absorption alters the post-extrusion pasting properties is less clear. For instance in **Table 6.5** it can be seen that barley flour, β -glucan, oat bran, psyllium have higher peak and final viscosities than the control sample (peak viscosity being a factor of starch gelatinisation whereas final viscosity normally being associated with the gel-forming nature of the hot samples). Thus the correlations of **Table 8.1** indicate that the higher the water absorption index of the sample the greater water movement during the extrusion process (especially between before and after the die face) thus the lower the degree of starch gelatinisation and hence the higher the pasting values of the product. From the experiments performed in this study there is insufficient data to determine if this is a cause of H2 or H3. Further work is therefore required to investigate water mobility during the extrusion process and evaluate the position of the water in the product during extrusion.

The manipulation of the pasting properties of the flour bases has an important implication on the potential digestibility of the starch within the mixture. Research by Chaplin (2003) and Tolstoguzov (2003; 2004) has clearly illustrated that in starch – polysaccharide systems the competition between the starch, protein and polysaccharide for available water is a limiting factor for starch gelatinisation and potential starch hydrolysis following ingestion.

The positive correlation between the number of texture peaks and the pasting properties of the products is complex and again related to water mobility and brittleness of the product. We have observed in **Figure 6.17** and **Figure 6.18** that the inclusion of fibre in the snack products can have dramatic effects on product hardness (**Figure 6.17**) and number of peaks associated with the fracture of the snack product (**Figure 6.18**). It is clear from these figures that we can not simply suggest that inclusion of any fibre increases or decreases hardness of a product. Fibres are diverse components and as such no fibre is the same, each one exhibiting their own characteristics, hence the degree of variability between and within

samples in **Figure 6.17** and **Figure 6.18** explains the lack of correlation between fibre levels and textural properties of the snack products as observed in **Table 8.1**. This observation can be seen in previous research, for instance a number of researchers have suggested that the addition of fibres has a negative effect on texture, making products harder and denser (Dehghan-Shoar *et al.*, 2010; Alavi, 2011). Other researchers have indicated that the inclusion of fibres can have a positive effect in making the texture of products more acceptable to consumers and reducing product hardness or creating more expanded, less dense products (Berglund *et al.*, 1994; Parada *et al.*, 2011). A few researchers have indicated that it is too simplistic to tar all fibres with the same brush (Brennan *et al.*, 2008b; Gordon, 1989)

In order to illustrate the fact that not all fibres behave the same further correlations were conducted on groupings of each fibre type. For instance, results obtained for β -glucan fibre at 5 %, 10 % and 15 % inclusion levels were analysed to see any correlations between components and physicochemical properties.

When evaluating the correlations shown in **Table 8.2** it can be observed that the majority of the values appear to be well correlated ($r \leq 0.80$), however for ease of understanding the significant correlations only those cells coloured in either blue or red are of significant importance at $P \leq 0.05$ level. The discrepancy between high correlations and low significance is a result of the high variations in sample values within the 5- 15 % of fibre inclusion range, and also a product of relatively low sample size ($n= 12$). In this discussion only significant correlations ($P \leq 0.05$) will be discussed.

Table 8.2. Pearsons correlation of snack food composition and physicochemical properties individual fibres.

β-glucan	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.999	0.958	-0.817	0.978	0.79	0.909	0.579
Starch		-0.971	0.786	0.965	-0.821	-0.886	-0.621
Exp			-0.617	0.876	0.933	0.75	0.78
Den				-0.92	-0.291	-0.983	-0.003
TH					0.643	0.976	0.395
TP						0.462	0.958
PV							0.186
FV							

Oat bran	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.507	0.779	0.4	0.998	0.667	0.751	0.366
Starch		0.145	-0.993	-0.556	0.304	-0.95	-0.988
Exp			-262	0.742	0.987	0.171	-0.298
Den				0.453	-0.416	0.906	0.999
TH					0.622	0.788	0.419
TP						0.008	-0.45
PV							0.89
FV							

Psyllium	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.954	-282	0.826	0.986	-0.386	0.99	0.994
Starch		0.558	-0.957	-0.889	0.631	-0.986	-0.982
Exp			-0.774	-0.116	0.996	-0.412	-0.389
Den				0.719	-0.828	0.896	0.885
TH					-2.06	0.953	0.96
TP						-0.493	-0.471
PV							0.998
FV							

Mushroom	Starch	EN/Ap	Den	TH	TP	PV	FV
Fibre	-0.951	0.702	N/A	N/A	N/A	-0.988	-0.972
Starch		-0.447	N/A	N/A	N/A	0.987	0.852
EN/Ap			N/A	N/A	N/A	-0.583	-0.849
Den				N/A	N/A	N/A	N/A
TH					N/A	N/A	N/A
TP						N/A	N/A
PV							0.924
FV							

Inulin	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.812	-0.852	0.988	-0.984	-0.98	-0.96	-0.787
Starch		0.387	-0.893	0.693	0.911	0.616	0.999
Exp			-0.76	0.933	0.733	0.965	0.348
Den				-0.943	-0.999	-0.904	-0.874
TH					0.929	0.995	0.662
TP						0.886	0.893
PV							0.582
FV							

Gatifolia	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.99	0.993	0.984	-0.96	0.998	0.999	0.996
Starch		-0.967	-0.999	0.911	-0.978	-0.994	-0.989
Exp			0.957	-0.986	0.999	0.989	0.98
Den				-0.896	0.971	0.99	0.969
TH					-0.976	-0.95	-0.932
TP						0.995	0.988
PV							0.999
FV							

Super gum	Starch	Exp	Den	TH	TP	PV	FV
Fibre	-0.973	0.903	0.646	-0.993	0.88	-0.938	0.876
Starch		-0.78	-0.804	0.993	-0.748	0.992	-0.963
Exp			0.254	-0.836	0.999	-0.696	0.582
Den				-0.73	0.206	-0.871	0.934
TH					-0.819	0.972	-0.926
TP						-0.66	0.541
PV							-0.999
FV							

Exp- expansion; Den- density; TH- texture hardness value; TP- texture number of peaks value; PV- pasting peak viscosity; FV- pasting final viscosity. Highlighted correlations significant at $p \leq 0.05$, blue representing a negative and red representing a positive correlation.

The statement that all fibres are different is exemplified by the correlations in **Table 8.2**. For instance if we look at the correlations of the amount of fibre to the physicochemical properties of the snack products we observe that fibre is negatively correlated to starch content in snack products containing β -glucan only. However none of the other correlations for β -glucan are significant at $P \leq 0.05$. Oat bran snack products show a significant positive correlation of increased fibre content to hardness of products, a negative correlation to starch content and product density, and a positive correlation between product density and final viscosity. This observation illustrates a relationship between the viscosity altering behaviour of oat bran and the texture of products. Similar

observations have been made by Tosh *et al.*, (2008) when utilising oat bran components in cereal food products such as muffins, cakes and biscuits.

Snacks with psyllium fibre inclusion show positive correlation between fibre content and final viscosity similarly indicating the relationship between the highly viscous nature of psyllium fibre and viscosity altering properties of the material (as already indicated by the water holding index and pasting properties in *Chapter 6*). Previous research has also confirmed that the inclusion of psyllium fibre can increase the viscosity of food products (Mariotti *et al.*, 2009; Vuksan *et al.*, 2011). In the psyllium rich samples expansion ratio was positively correlated to the number of peaks observed when the product is compressed using the texture analyser. There was also a significant positive correlation between peak viscosity and final viscosity. This last correlation would be expected in relation to the viscosity altering nature of psyllium fibre. No significant correlations were observed within the mushroom samples. Inulin enriched samples showed positive correlations between starch content and final viscosity and hardness of products with peak viscosity, whereas a negative correlation existed between density and the number of peaks observed during compression of the product. These observations could be related to the fact that inulin is highly water soluble (as evidenced by the high water solubility index value **Figure 6.10** and **Figure 6.11**) so then starch content was the significant contributor to viscosity and texture in these products rather than inulin content. Gatifolia fibre content was positively correlated to expansion ratio, number of peaks during texture analysis, peak viscosity and final viscosity (the latter four properties being positively correlated with each other as well).

Chapter 6 illustrated that inclusion of gatifolia resulted in increased expansion ratio and reduced product density and product hardness. These correlations are consistent with the observations in *Chapter 6*. The significant negative correlation between starch content and peak viscosity is interesting as conventional understanding indicates that a higher amount of starch should give rise to increased pasting properties. Further research is required to identify whether this is a product of small sample size or whether there is another factor manipulating this relationship. Super gum inclusion shows a significant negative correlation with fibre level and hardness of the product and peak viscosity to final viscosity, whereas positive correlations exist between starch content and hardness and peak viscosity, as well as between expansion ratio and the number of peaks recorded during compression of the product. No previous research has been found on the effect of gatifolia

or super gum on extruded snack product properties, so this element of the research provides a starting point in the exploration of these gum components in food extrusion.

8.3 How fibre effects the digestibility of starch

Ungelatinised starches are not easily digested by humans and are nutritionally regarded as potentially resistant starches (Holm *et al.*, 1988; Butterworth *et al.*, 2011). Although the extrusion process cooks and melts the raw material within the extruder, water levels are normally well regulated and may prevent complete gelatinisation (Bornet, 1993; Chessari & Sellahewa, 2001). The digestibility of starch may be improved by the extrusion process due to partial gelatinisation and fragmentation of starch attributed to the mechanical shearing effect of the extruder on starch granules (Wang *et al.*, 1993). Extrusion may be considered as a method to initiate the pre digestion of starch, consequently it is not surprising therefore that extruded snack products tend to be regarded as having high GI status (Capriles *et al.*, 2009; Onwulata *et al.*, 2010). Their high GI status is largely due to the low NSP or DF composition of such snack products combined with the high carbohydrate content of the cereals. However it is also related to the extrusion process which alters the chemical composition of the food product, and the digestibility of the starch within the carbohydrate food products. **Section 6.2** of this thesis illustrated this fact that extruded snack products showed a higher overall starch digestibility than the raw (un-extruded) sample mixes.

This thesis aimed to investigate the complex relationship between different DF components and starch digestion. Thus for the remainder of this section the results of *Chapter 6* (using an *in vitro* digestion process) will be discussed. **Figures 6.23-6.30** illustrated the potential incremental rate of release of glucose over a 120 min digestion process, converting reducing sugar release to potential glucose units. In examining the extruded samples it can be observed that DF inclusion has a significant effect in reducing the amount of starch degradation (and potential glucose release) compared to the control sample.

What is of great interest is that this effect is not dose responsive in all of the fibre samples studied. For instance whilst inclusion of β -glucan, oat bran, gatifolia and super gum all show a significant reduction in starch digestibility compared to the control sample, the reduction in digestibility is similar for all levels of inclusion. If we look at β -glucan as the

example of this phenomenon, we can observe from **Figure 6.23** that inclusion of β -glucan at 5 % level reduces the final glucose reading at time 120 by a 18 % from the control sample value, adding more β -glucan did not significantly alter the potential starch digestibility of the 10 or 15 % β -glucan samples (remaining at the 18 – 20 % reduction level). Similar observations were found for snacks containing oat bran (~ 15 % reduction irrespective of inclusion level), gatifolia (~ 17 % reduction irrespective of inclusion level), and super gum (~22 % reduction irrespective of inclusion level). Previous research from, our laboratory (Brennan *et al.*, 2008a,b) and others (Chillo *et al.*, 2011; Willis *et al.*, 2011), has indicated similar patterns in that in some fibre enriched food products the potential glycaemic response lowering effect is not dose responsive. Indeed some authors have reported that particular fibres under certain conditions may increase starch digestibility leading to an increased glycaemic response (Foster-Powell *et al.*, 2002; Willis *et al.*, 2011). In this study inclusion of psyllium fibres at 5–15 % levels led to a dose responsive reduction of between 16 % (at 5 % inclusion level) to 30 % (at 15 % inclusion level) in potential glucose release from the snack products (**Figure 6.25**). Inclusion of mushroom waste material at 5 % led to a reduction in potential glucose release of 6 % whereas at 15 % inclusion rate this reduction was 27 %. Inulin showed a step wise reduction in potential glucose release of 10 % (at 5 % inclusion level) to 16 % (at 15 % inclusion level).

Whilst overall starch digestibility is of interest in determining the nutritional quality of foods, when discussing the relevance of food processing to the glycaemic impact of foods, one of the most important concepts to think about is the difference between rapidly digestible, slowly digestible and resistant starch fractions. The rapidly digestible fraction (starch digested between 0-20 min) can lead to a rapid rise in blood sugar levels (hence a rapid glycaemic response) once a food item is ingested. This “spike” in blood sugar levels has several negative effects for the health of diabetics and also a non-diabetic individual (Jenkins *et al.*, 1987; Livesey *et al.*, 2008). Most of the early research conducted by Jenkins *et al* was aimed at developing strategies to attenuate this glycaemic response through the manipulation of the rate of starch digestibility (Jenkins *et al.*, 1978; 1983; 1987; 1997; Fairchild *et al.*, 1996).

Work from our laboratory (Brennan, 2005; Brennan *et al.*, 2008a) has illustrated that by manipulation of product composition it is possible to increase the amount of slowly digestible starch components (digestibility between 60-120 min in an *in vitro* procedure) in processed foods, hence reduce the rate and extent of predicted glycaemic response. For

instance, the utilisation of wholemeal flour in extruded snack products, instead of refined flour components, has been shown to reduce the amount of rapidly digestible starch components and increase the amount of slowly digestible starch in extruded breakfast cereal products (Brennan *et al.*, 2008a,b). Mishra *et al.*, (2008) have also illustrated that differing the processing techniques employed in the cooking of potato can increase the amount of slowly digestible starch in a potato meal, hence reduce the potential glycaemic impact of such a product. Particle size of the carbohydrate source can also impact on the digestibility of the starch fraction. This has been given as one of the reasons why chickpea and pasta grade flours generally produce lower glycaemic responses compared to finely milled bread wheat flour (Hardacre *et al.*, 2006).

Figure 6.33 indicates the incremental glucose release during the *in vitro* digestion of all the fibre enriched samples. β -glucan, psyllium, mushroom waste and gatifolia inclusion all had a dose response in the potential reduction of glucose release. This can be further illustrated by their step wise reduction in standardised AUC (**Figure 6.34**). Research studies conducted in the 1980's and 1990's indicated that certain fibres alter postprandial glycaemia in both healthy and diabetic subjects (Potter *et al.*, 1981; Gatti *et al.*, 1984; Bourdon *et al.*, 1999). This effect on blunting glycaemic increase, together with a delaying of gastric emptying, and enhanced satiety have been associated with the consumption of soluble fibres (Slavin, 2009; Guérin-Deremaux *et al.*, 2011). The current study expanded this area through the meaningful findings related to the rate of starch digestion following the *in vitro* experiments conducted.

In agreement to other studies carried out *in vivo* and *in vitro* (Wolever & Jenkins, 1986; Casiraghi *et al.*, 1992) this work indicates that incorporation of certain fibres may lead to significantly lower glucose response than an equivalent amount of carbohydrate in form of controlled non fibre enriched products. The reduced rate of starch digestibility in products containing fibres is proposed to be the result of a combination of rate-limiting factors: increased product viscosity; reduced starch swelling and rate of amylose leaching out of the granules; formation of a layer coating starch granules, which may act as a barrier between starch and α -amylase; lengthened path between starch granules and α -amylase; potential inhibition of α -amylase by fibres.

In order to attempt to understand the inter-relationships between fibre content and the starch digestibility quality of foods a Pearson's correlation was conducted on all data from the samples manufactured during the first stage of the research (**Table 8.3**). Examining the collated data from all samples a negative correlation exists between the amount of fibre in the samples and all of the starch digestibility determinants. Significant negative correlations exist between starch content and rapidly digestible starch (glucose release between 0-20 min of the digestion process) as well as overall AUC. This supports the observation that incorporation of fibre into the extruded snack products leads to a reduction in starch digestibility and hence potential glucose release during an *in vitro* digestion process.

Table 8.3. Pearsons correlation of snack food composition and starch digestibility properties.

	0-20	0-60	60-120	AUC
Fibre	-0.413	-0.385	-0.046	-0.425
Starch	0.551	0.638	0.108	0.62
Exp	-0.52	-0.476	0.23	-0.414
Den	-0.066	-0.087	-0.063	-0.053
TH	0.349	0.563	0.332	0.467
TP	-0.414	-0.34	0.179	-0.312
PV	-0.372	-0.278	0.332	-0.246
FV	-0.332	-0.332	0.327	-0.29
0-20		0.88	-0.287	0.798
0-60			-0.114	0.869
60-120				0.059

Exp- expansion; Den- density; TH- texture hardness value; TP- texture number of peaks value; PV- pasting peak viscosity; FV- pasting final viscosity; 0-20 - *in vitro* digestion reading after 20 mins; 0-60 - *in vitro* digestion reading after 60 mins; 60-120- *in vitro* digestion reading between 60-120 mins; AUC - *in vitro* area under the curve value. Highlighted correlations significant at $p \leq 0.05$, blue representing a negative and red representing a positive correlation.

The starch content of the product is seen to be significantly positively correlated to glucose release at the 0-20 and 0-60 min stages of digestion as well as overall AUC. This is unsurprising as simplistically the amount of glucose released during the digestion process is related to the amount of available starch in the product. What is of interest in these correlations is that the degree of product expansion is significantly negatively correlated to digestion processes, whereas the hardness of the products is positively correlated to glucose release at the 0-60 min stage of digestion as well as overall AUC.

Table 8.4 shows that as with the physicochemical correlations, not all fibres behaved in the same way with regards to the correlations in terms of starch digestibility. For instance, samples which had β -glucan inclusions showed a significant negative correlation in terms of fibre and glucose release at the 0-20 min stage of the *in vitro* process, whereas oat bran samples showed a positive correlation between fibre levels and glucose released between the 0–60 min stage of the *in vitro* process. Snack products with added oat bran were the only samples to show a positive correlation between fibre levels and glucose released. Psyllium containing products showed a significant negative correlation between fibre content and glucose release at both the 0-20 min and 0–60 min stages of the *in vitro* process. The differences between the oat bran samples and the psyllium samples was one of the major reasons why these two products were selected for further investigation in the *in vivo* trial.

Table 8.4 Pearsons correlation of individual fibre enriched snack food composition and starch digestibility properties.

β -glucan	0-20	0-60	60-120	AUC
Fibre	-0.996	0.759	-0.877	-0.134
Starch	0.999	-0.783	0.851	0.082
Exp	-0.979	0.908	-0.702	0.156
Den	0.765	-0.23	0.994	0.681
TH	-0.956	0.594	-0.958	-0.339
TP	-0.839	0.998	-0.397	0.502
PV	-0.87	0.405	-0.997	-0.535
FV	-0.647	0.974	-0.116	0.73
0-20		-0.803	0.833	0.049
0-60			-0.339	0.556
60-120				0.594

oat bran	0-20	0-60	60-120	AUC
Fibre	0.463	0.999	-0.671	0.53
Starch	-0.999	-0.514	0.979	0.809
Exp	-0.194	0.774	-0.058	0.988
Den	0.998	0.408	-0.948	-0.408
TH	0.514	0.999	-0.713	0.629
TP	-0.352	0.661	0.106	0.978
PV	0.933	0.756	-0.994	0.017
FV	0.994	0.373	-0.936	-0.442
0-20		0.47	-0.968	0.343
0-60			-0.627	0.667
60-120				0.097

Psyllium	0-20	0-60	60-120	AUC
Fibre	-0.998	-0.999	0.946	-0.981
Starch	0.993	0.966	-0.804	0.994
Exp	0.222	0.324	0.044	0.462
Den	-0.79	-0.85	0.599	-0.919
TH	-0.994	-0.977	0.987	-0.935
TP	0.31	0.41	-0.047	0.541
PV	-0.98	-0.996	0.892	-0.998
FV	-0.985	0.998	0.903	-0.997
0-20		0.994	-0.964	0.967
0-60			-0.931	0.989
60-120				-0.866

Mushroom	0-20	0-60	60-120	AUC
Fibre	-0.987	-0.992	0.798	-0.964
Starch	0.888	0.904	-0.945	0.999
EN/Ap	-0.808	-0.787	0.131	-0.487
Den	N/A	N/A	N/A	N/A
TH	N/A	N/A	N/A	N/A
TP	N/A	N/A	N/A	N/A
PV	0.95	0.96	-0.882	0.994
FV	0.997	0.994	-0.635	0.875
0-20		0.999	-0.69	0.908
0-60			-0.715	0.922
60-120				-0.93

Inulin	0-20	0-60	60-120	AUC
Fibre	-0.464	-0.999	-0.644	-0.979
Starch	-0.14	0.837	0.969	0.915
Exp	0.859	0.829	0.149	0.727
Den	-0.32	-0.994	-0.756	-0.999
TH	0.617	0.975	0.495	0.926
TP	0.28	0.988	0.782	0.998
PV	0.694	0.947	0.403	0.883
FV	-0.182	0.813	0.979	0.897
0-20		0.425	-0.379	0.272
0-60			0.676	0.987
60-120				0.787

Gatifolia	0-20	0-60	60-120	AUC
Fibre	-0.98	-0.987	0.186	-0.659
Starch	0.941	0.999	-0.324	0.574
Exp	-0.996	-0.963	0.071	-0.764
Den	0.929	-0.999	0.071	-0.764
TH	0.997	0.903	0.096	0.861
TP	-0.991	-0.974	0.121	-0.731
PV	-0.972	-0.992	0.22	-0.659
FV	-0.959	-0.997	0.271	-0.681
0-20		0.935	0.015	0.817
0-60			-0.341	0.559
60-120				0.589

Super gum	0-20	0-60	60-120	AUC
Fibre	-0.988	-0.998	0.781	0.22
Starch	0.997	0.985	-0.903	-0.438
Exp	-0.827	-0.876	0.435	-0.222
Den	-0.754	-0.689	0.981	0.887
TH	0.999	0.998	-0.848	-0.332
TP	-0.798	-0.851	0.39	-0.27
PV	0.98	0.956	-0.949	-0.545
FV	-0.939	-0.992	0.985	0.664
0-20		0.996	-0.867	-0.366
0-60			-0.816	-0.276
60-120				0.781

Exp- expansion; Den- density; TH- texture hardness value; TP- texture number of peaks value; PV- pasting peak viscosity; FV- pasting final viscosity; 0-20 - *in vitro* digestion reading after 20 min; 0-60 - *in vitro* digestion reading after 60 min; 60-120 - *in vitro* digestion reading between 60-120 min; AUC- *in vitro* area under the curve value. Highlighted correlations significant at $p \leq 0.05$, blue representing a negative and red representing a positive correlation.

Evaluating each fibre sample separately, correlations in the β -glucan containing samples showed negative correlations with fibre and 0-20 min digestion, and peak viscosity and 60-120 min digestion. These negative correlations potentially illustrate a connection between fibre viscosity development and reduction in glucose release during digestion as observed by Jenkins *et al.*, (1978). Positive correlations were observed between starch and 0-20 min digestion process, density and 60-120 min digestion and the number of peaks observed during compression of the samples with the 0-60 min stage of digestion. These positive correlations could be related to the role of starch in altering the textural properties of snack products as observed by Hardacre *et al.*, (2006) who demonstrated a connection between higher starch levels and more brittle final product.

Oat bran containing samples showed a negative correlation between starch and digestion at 0-20 min stage and peak viscosity and digestion at 60-120 min. Positive correlations existed between fibre and digestion at stage 0-60 min, product density and digestion stage at 0-20 min, product hardness and digestion stage at 0-60 min as well as final viscosity and digestion stage at 0–20 min. These correlations illustrate a potential relationship between high fibre contents inducing high viscosity levels which in turn alter water mobility and hence product density / hardness, which in turn affects overall glucose release.

Psyllium containing samples showed negative correlations between fibre and digestion values at 0-20 and 0-60 min stages, peak viscosity and digestion at the 0-60 min stage as well as peak and final viscosity with overall AUC. These correlations are supportive of the digestion profiles shown in *Chapter 6* illustrating that increasing levels of psyllium fibre reduced the potential glucose release from such snack products, as well as supporting the previous discussion suggesting that fibre and water interactions play a major contribution to glucose release during starch digestion.

Correlations in terms of the mushroom waste containing products were mainly dominated by starch content in that starch was positively correlated with AUC and pasting viscosity values (peak viscosity and final viscosity) were correlated to starch digestion rates (AUC and the digestion stages at 0-20 and 0-60 min). Increasing fibre content was seen to have a negative correlation with the glucose released at the 0–60 min digestion stage. This supports the observation in *Chapter 6* illustrating the potential reductions in glucose release and AUC with higher levels of mushroom waste product.

Inulin also showed a negative correlation between fibre content and glucose released at the 0-60 min stage, as well as density of the product to overall AUC. This suggests that fibre inclusion has both an effect on product density and starch digestibility. In a previous paper (Brennan *et al.*, 2008b) a similar observation is recorded, in that increased inulin content of breakfast cereals was related to increased expansion and a reduction in product density, whilst being related to a reduction in glucose released from the breakfast cereal products studied.

8.4 Effect of inclusion of psyllium and oat bran material at 15 % inclusion level on the physicochemical and nutrition properties of (phase two) snack products as evaluated by both *in vitro* and *in vivo* determinations

On completion of the initial screening of the different fibre sources and levels of fibre inclusion, psyllium fibre and oat bran fibre material were selected as being two materials which behaved differently to each other. Oat bran and psyllium represent two relatively well studied fibres in terms of their use in glycaemic modulating food products (Anderson *et al.*, 1999; Regand *et al.*, 2011). Both of these fibres have been shown to have a postprandial glycaemic reducing potential and are freely available for use as health additives from health food stores. Researchers have investigated the role of different dietary fibres in model food systems in the modulation of glycaemic response either by hypothesising from *in vitro* starch digestion methods, or by invasive *in vivo* glycaemic response trials (Woolnough *et al.*, 2008). However no previous research has been conducted comparing the postprandial glucose reducing potential of these dietary fibres in extruded snack food products. The purpose of this part of the study was to evaluate such a potential using both *in vitro* starch degradation methodology and conventional *in vivo* glycaemic response procedures in healthy subjects.

The effect of both oat bran and psyllium fibres in reducing postprandial blood glucose levels have been studied by numerous scientists in clinical trials. Indeed several researchers have attempted to investigate the *in vivo* response of individuals consuming oat bran rich food products (Granfeldt *et al.*, 1992; Tosh *et al.*, 2008; Regand *et al.*, 2011). Such works have shown that oat bran can contribute to lowering the glycaemic impact of foods potentially through the effect of β -glucans contained in oat bran altering the rheological nature of food digesta (Dikeman & Fahey, 2006; Regand *et al.*, 2011). In a similar vein researchers have endeavoured to explain observed reduction in glycaemic responses of individuals after ingestion of psyllium extracts through potential viscosity related properties inhibiting starch degradation (Florholmen *et al.*, 1982; Wolever *et al.*, 1991b). This section attempts to compare the effect of these two food ingredients in manipulating the potential and real glycaemic response of foods using *in vivo* and *in vitro* procedures. Whilst there appeared to be no direct correlation between the measurements recorded after *in vivo* and *in vitro* significant observations can be made.

Both psyllium and oat bran snacks showed a reduction with *in vitro* digestion compared with the control. The addition of psyllium fibre to the snack products resulted in a greater

than 15 % reduction of glucose production over 120 min. Addition of oat bran to the snack products resulted in a 5.4 % reduction in glucose produced. Similar results have been obtained by a number of researchers investigating both soluble and insoluble dietary fibres, in such reports the authors have explained the effect of fibre in reducing starch degradation on the possibility of fibres coating starch granules and inhibiting enzyme penetration (Brennan *et al.*, 1996; Al-Rabadi *et al.*, 2011) and the possibility that the viscous nature of fibres affects the efficiency of enzyme functionality (Braaten *et al.*, 1991; Flammang *et al.*, 2006).

In terms of product physicochemical characteristics the inclusion of oat bran at 15% to the snack products yielded products with an increased product density and decreased product expansion ratio compared to the control product. The decrease in product density with increased oat bran content is consistent with the results observed in the initial trial and also in agreement with research by Tosh *et al.*, (2010). Tosh *et al.*, (2010) indicated that the viscous nature of oat bran reduced product expansion and increased product density compared to control products. The textural characteristics of the oat bran containing products showed a non-significant increase in product hardness (which could be related to product density). Psyllium fibre inclusion at 15% also showed an increased product density compared to the control and a related increase in product hardness, however the expansion ratio of the product was not significantly different to the control sample. The density altering behaviour of the oat bran and psyllium fibres could be related to the viscosity altering nature of the products as discussed previously. This phenomenon is also reflected in the significant reduction in water solubility index values of the extruded samples containing oat bran and psyllium (**Figure 7.2**) together with the differences between the fibre samples and the control product in terms of water absorption index (**Figure 7.1**)

Both psyllium and oat bran snacks showed a reduction with *in vitro* digestion compared with the control (**Figure 7.3**). The addition of psyllium fibre to the snack products resulted in a greater than 15 % reduction of glucose production over 120 min (~ 20 % reduction observed). Addition of oat bran to the snack products resulted in a 5.4 % reduction in glucose produced. This result was not expected as one would assume that if an inert filler was incorporated into a starch food system a 15 % reduction in starch digestion products would result on the basis of straight forward mass balance reduction. As such the results

suggest that inclusion of oat bran may be leading to an increase in glucose release (proportional to the starch content of the sample).

As discussed previously, numerous authors have reported results in terms of fibre reducing glucose release obtained in both soluble and insoluble dietary fibres. In such reports the authors have explained the effect of fibre in reducing starch degradation on the possibility of fibres coating starch granules and inhibiting enzyme penetration (Brennan *et al.*, 1996; Anderson *et al.*, 1999; Brennan *et al.*, 2002; Brennan, 2005; Granfeldt *et al.*, 2008) and the possibility that the viscous nature of fibres affects the efficiency of enzyme functionality (Jenkins *et al.*, 2002; Flammang *et al.*, 2006; Kendall *et al.*, 2008; Aravind *et al.*, 2012). It is probable that inclusion of psyllium fibre to the extruded snack products is acting in this viscous related manner in modulating starch digestion process. Indeed these results echo those illustrated in **Figure 6.32** and

Figure 6.33 in the initial stages of the thesis research.

As described earlier, the absolute glucose release value for the oat bran snack was still a significant reduction compared to the control snack (as illustrated in the incremental AUC values shown in **Figure 7.5**) it was less than the 15 % reduction to be expected if the replacement value of oat bran to potential carbohydrate from the control recipe was to be taken into account. One possible reason for this may be that the extrusion process has depolymerised the β -glucans in the oat bran, making them less viscous and limiting their effectiveness in regulating starch digestion through viscosity mediated events. Regand *et al.*, (2011) observed the effect of processing on the relationship between oat β -glucans and glycaemic effects. Further analysis investigating the role extrusion plays on the molecular structure of the ingredients used in this study is required to elaborate on whether extrusion processing affected the ability of oat bran and psyllium to manipulate glycaemic response.

In the *in vivo* blood glucose experiment the psyllium snack produced a much lower AUC than the control snack, oat bran snack and glucose drink (**Figure 7.7**). Again, this effect could be related to the manner in which dietary fibre reduces glucose release either through binding the starch to prevent amylolytic degradation or by altering the overall viscosity of the digesta limiting water mobility and hence enzyme accessibility (Brennan *et al.*, 1996; Brennan *et al.*, 2008b; Regand *et al.*, 2011). According to the results presented in this section the oat bran snack seemed to produced much higher peak than expected if

comparing the results of similar studies using β -glucan rich oat bran fractions in muffins and other food systems (Izydorczyk *et al.*, 2008; Tosh *et al.*, 2008; Regand *et al.*, 2011). This observation could be a result of the extrusion process depolymerising the β -glucan and hence reducing its' ability to attenuate the starch-enzyme digestion process. Having produced a higher peak, the blood glucose level after consuming the oat bran snack did not fall back to the fasting blood glucose within the 120 min of the experiment. This is a thought provoking observation in that it suggests that the oat bran may have a role in affecting the overall digestion rate of starch and thereby manipulating the glucose release rate. The indication is that oat bran allows for protracted release of glucose (**Figure 7.6**) that is sustained over time hence leading to an elevated AUC. However at the same time the oat bran sample would delay the occurrence of the “dip” during a postprandial glucose plot hence potentially flattening out troughs in the postprandial blood glucose curve. Any flattening out of the postprandial glucose response may have an impact on raising the level of feeling full for an individual thereby contributing to greater satiety ratings. Such an observation is in line with previous researchers studying a diverse range of fibres and food combinations (Brennan, 2005; Brennan *et al.*, 2008b; Izydorczyk *et al.*, 2008; Tosh *et al.*, 2008; Roder *et al.*, 2009; Butterworth *et al.*, 2011).

In order to attempt to understand the interrelationships between fibre content and the starch digestibility value of the final snack food produced (as well as any potential relationship between *in vitro* and *in vivo* digestibility determinations) a Pearson's correlation was conducted on all data from the samples manufactured for the *in vivo* trial (**Table 8.5**).

Table 8.5 Pearson's correlation of physicochemical components and starch digestibility properties of the control, and fibre enriched snack products.

	WSI	Den	Exp	TH	TP	PV	FV	0-20	20-60	60-120	In vitro AUC	In Vivo AUC
WAI	-0.75	0.22	0.3	0.77	0.68	0.97	0.99	-0.81	-0.47	0.98	-0.85	-0.93
WSI		0.48	-0.68	-0.2	-0.99	-0.89	-0.84	0.99	-0.23	-0.86	0.99	0.45
Den			-0.97	0.66	-0.71	-0.99	0.17	-0.61	0.87	0.2	-0.55	0.35
Exp				-0.46	0.86	-0.98	0.07	0.4	-0.96	0.03	0.33	-0.56
TH					0.05	-0.97	0.85	-0.99	0.21	0.86	-0.99	-0.47
TP						-0.91	0.56	-0.11	-0.96	0.53	-0.19	-0.9
PV							0.99	-0.88	-0.33	0.99	-0.92	-0.87
FV								-0.99	-0.93	-0.53	-0.99	-0.86
0-20									-0.14	-0.9	0.99	0.53
20-60										-0.3	-0.07	0.76
60-120											-0.93	-0.85
In vitro Auc												0.6

Exp- expansion; Den- density; TH- texture hardness value; TP- texture number of peaks value; PV- pasting peak viscosity; FV- pasting final viscosity; 0-20- *in vitro* digestion reading after 20 min; 0-60- *in vitro* digestion reading after 60 min; 60-120- *in vitro* digestion reading between 60-120 min; AUC- *in vitro* area under the curve value. Highlighted correlations significant at $p \leq 0.05$, blue representing a negative and red representing a positive correlation.

The final set of correlations cover the relationship between the physicochemical properties of the 15 % fibre enriched snack products to the glycaemic and potential glycaemic responses of these products. Some significant correlations can be seen indicating the main components associated with mediating the glycaemic response of fibre enriched extruded snack products. For instance the correlations support the claims made in previous sections that water and water mobility plays a significant role in both the physical nature and nutritional value of extruded snack products. The WAI is positively correlated to both the peak and final viscosity values of the extruded samples. This clearly supports the suggestion that the ability of a food material to absorb and hold on to water contributes to the overall viscosity of that product and goes hand in hand with the idea of using fibre components as gums and stabilisers. What is subsequently interesting to note is that the WAI is negatively correlated to the release of glucose at the 0-20 stage of an *in vitro* digestion process and also the AUC values for both *in vitro* and *in vivo* glucose responses. This lends significant weight to the suggestion that the limiting factor in terms of starch digestibility and glucose release is the viscous nature of the food product. Highly viscous products (products capable of absorbing large amount of water) can therefore lead to a reduction in starch digestion. The inverse relationship is also observed in that the greater the solubility of the product the higher the degree of starch digestibility (certainly when considering the correlation between WSI and *in vitro* AUC). Again, this appears to be viscosity driven in that significant negative correlations exist between WSI and the peak and final viscosity of extruded samples. Simplistically, the greater the solubility of the product the greater the ease of the product (starch and fibre) to be dispersed in an aqueous solution and hence the greater the opportunity for water mobile enzymes to locate substrate during digestion. The positive correlation between WSI and starch digestibility at 0-20 min stage of the *in vitro* process adds weight to this suggestion.

Table 8.5 illustrates that the density of an extruded product is negatively correlated to the expansion ratios. Interestingly for these particular samples density is positively correlated to the starch digestibility at 20-60 min stage. Thus in these samples the denser the product the greater the glucose release after 20 min of digestion, however no significant correlation

can be associated between density and either *in vitro* or *in vivo* AUC values. Expansion ratio is positively correlated with the number of peaks observed during compression but negatively correlated to peak viscosity and the glucose released during the 20-60 min stage (possibly related to the viscosity altering effects of psyllium fibre). The correlations between the texture characteristics and pasting and digestion properties of the snack products also appear to be related to the viscosity altering nature of the products. For instance both the hardness of the products and the number of peaks of the samples (crunchiness of the products) are negatively correlated to the peak viscosity of the product either derived from starch gelatinisation or through the WAI of the fibre components. The subsequent negative correlations to glucose release at the 0-20 and 20-60 min digestion stages are related to the AUC values for both the *in vitro* and *in vivo* glycaemic response evaluation. This relationship between product viscosity, water mobility and digestibility is further illustrated by the negative correlations between peak and final viscosity and both the *in vitro* and *in vivo* AUC values.

A positive correlation was also observed between the glucose released between 0-20 min stage (readily digestible starch fraction – RDS) of the *in vitro* process and total *in vitro* AUC value (but not *in vivo* AUC). Although the correlation between *in vitro* AUC determination and *in vivo* AUC determination was not significant ($p \leq 0.098$) a significant negative correlation was observed between the glucose released between the 60-120 min stage of the *in vitro* digestion procedure (slowly digestible starch fraction – SDS) and both *in vitro* and *in vivo* AUC values. Thus it is possible to conclude that there is a relationship between increased SDS and reductions in glycaemic response postprandially. Mechanisms to manipulate the proportion of RDS and SDS (effectively the rate and extent of starch digestion) are therefore important in the manufacture of low glycaemic response foods.

It is clear from the correlations as summarized in **Table 8.5** (together with the results observed in *Chapters 6* and *7*) that the use of NSP and DF to alter the glycaemic impact of foods may be due to the viscosity altering behavior of the polysaccharide. Non-starch polysaccharides, and DFs, have been used for many years to modulate the extent of starch swelling, gelatinisation and hence carbohydrate digestibility. For instance, clinical studies have shown that diets rich in soluble fibre such as guar gum, pectin and sugar beet fibre, result in lower postprandial blood glucose and insulin levels (Jenkins *et al.*, 1987). Soluble fibres from oats and barley, create gelatinous formations within the stomach which appear to delay gastric emptying and enzymatic digestion (Jenkins *et al.*, 1978), whereas insoluble

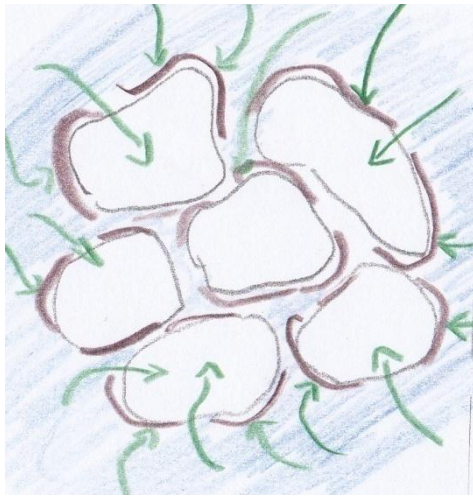
fibres have little effect on gastric emptying and no effect on glucose absorption. This may in part explain why high fibre diets alone are not necessarily synonymous with low glycaemic index foods (Jenkins *et al.*, 1983).

The hydration dynamics of fibre has been linked to the functionality of starches (Parvathy *et al.*, 2007) with much attention focused on galactomannan conformations and the structure of entangled networks these components form). However the effect fibres have on the viscosity of products may not be the sole mode by which polysaccharides work in controlling the glycaemic impact of carbohydrate rich food products (Brennan *et al.*, 1996). For instance, the viscosity altering properties of guar gum have been investigated in relation to the potential glycaemic effect of extruded snack products.

More recently research from the Kings College group (Butterworth *et al.*, 2011) has suggested that the main contribution to the potential glycaemic response of a product is related to substrate limiting events. For instance Butterworth *et al.*, suggest that the differentiation of starch into SDS and RDS fractions is erroneous and that all starch granules (and products) have the same glycaemic potential with the rate of reaction being limited by the accessibility of amylolytic enzymes to starch components. The research presented in this thesis contributes to the collection of data suggesting that modulation of the glycaemic response of individuals to starchy foods is largely dependent on water mobility and its effects on starch gelatinisation events. To this end, water is the key component to investigate in future research. If fibre alters the viscosity of the digesta (as is suggested by the correlations between peak and final viscosity and fibre components) then this will have an impact on the rate of starch gelatinisation (available substrate) as well as substrate – enzyme kinetics (mobility of enzymes in a viscous solution, in order to react with the substrate).

Thus one can summarise the possible mechanisms by which DF alter the starch digestion process into 5 possible scenarios. Although these are simplistic one can visualize them as shown in **Figure 8.1**.

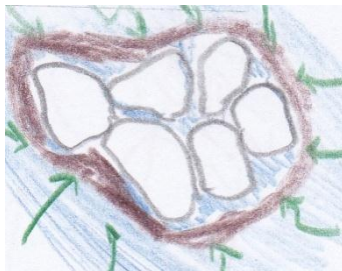
Mode 1: Some DF's associate with the starch granule surface and "coat" the granule impeding enzyme binding



Mode 2: Fibre hydrates to form a viscous continuum in which starch is interspersed limits enzyme mobility (free water) and accessibility



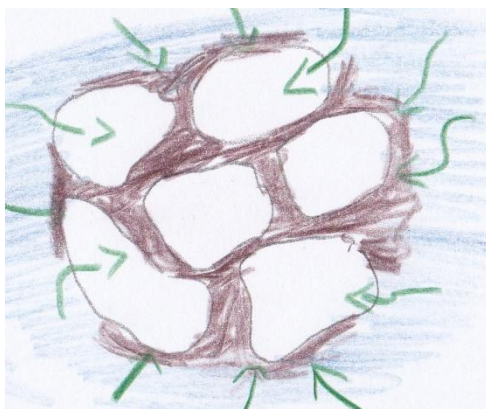
Mode 3: DF's compete with starch for water and hydrate preferentially, reducing free water available for starch gelatinisation, limiting granule disruption



Mode 4: Fibre is thermodynamically incompatible with starch and forms discrete hydrated structures which disrupt starch-protein structure and render starch granules more accessible to enzyme action



Mode 5: Fibre hydrates forming a gelatinous mass impermeable to enzymes this delaying the degree of enzyme degradation until disruption of the fibre continuum



Starch granules represented as opaque shapes in a solution (blue-water) with DF (brown) manipulating enzyme (green) accessibility. Diagrammatic representation only and not drawn to scale.

Figure 8.1. Mechanisms by which DF could affect starch digestion.

The combination of previous research and the results from the current experiments reported in this thesis suggest that the role of water and food structure is important in determining the mechanisms of starch digestibility. The over-all effect of DF on starch digestion is likely to be a combination of all 5 modes of action represented above. However, the determination of which mode of action has the predominant effect in the modulation of starch digestion and hence glucose release requires a closer look at the mobility of enzymes within the “free” and “bound” water in food structures.

Chapter 9

Conclusion, Limitations and Future Research Directions

9.1 Recap on original aims of the research project

The aims of this project were to utilise material high in fibre into RTE snack products, so as to improve the nutritional quality of such food products. Specifically to:-

1. Evaluate suitable commercially available dietary fibres and fibres in the co-products of food waste stream production for inclusion into fibre rich extruded snack products.
2. Analyse and determine the relationship between the incorporation of fibre rich materials into extruded food products and their effects on the physicochemical properties of the food products and sensory analysis characteristics.
3. Evaluate the effect of extrusion technology on the nutritional composition of the extruded snack products (namely starch digestibility and glucose release).
4. Compare and contrast the glycaemic response of fibre rich products recorded using *in vivo* and *in vitro* analysis.

9.2 Ph. D. limitations

At the outset of this project it was the intention to screen suitable DF for carrying forward into snack production.

The product made with 65 % barley flour i.e. complete wheat flour replacement showed that it was high in DF and β -glucan, it would have been interesting to carry this forward as a potential whole grain snack however it was only possible to carry forward two experimental products and a control due to time and economic restraints. Psyllium showed the greatest potential to attenuate glycaemic response consequently this was a definite

choice. As psyllium is not a waste product it was considered important that the other DF carried forward should be a waste product consequently oat bran (waste from oat milling industry) was included in phase two rather than the barley sample.

Similarly taking a mushroom waste snack forward to phase two could have yielded much more information, however inclusion at 15 % had not been successful in terms of creating a suitably expanded product. During phase one we attempted to use mushroom waste at 15 % but our attempts at manipulating the extrusion parameters to derive a consistent product failed due to inexperience in the people involved in extrusion at MMU. For this reason there are some gaps in data recorded for the 15 % mushroom waste product. Additionally preliminary sensory feedback established negative comments regarding the taste of mushroom products.

If time and money were no object, further development of the barley flour samples and the mushroom waste samples at levels of 15 % inclusion would be desirable.

Analysis of the samples for β -glucan and DF composition is not complete for all samples produced. Both procedures are time consuming and although the method is presented as a kit form, no other member at MMU had used these approved method kits before. As such there was a degree of learning which meant a number of weeks perfecting the technique. As the barley flour and the mushroom waste material had high levels of β -glucan in them they generated spurious results at times. After discussing this fact with Dr Barry McCleary of Megazyme International (the kit designer) it was decided to move on to the next phase of the experiments as they were not crucial in the choice for which samples to progress to phase two.

The intervention trial itself had various limitations. Due to recruiting staff and students who had offices on site they could leave the area set aside for them to return to their desks to work. Although they understood the need not to consume food for the duration of the trial, they may have been more active than the participants who remained sedentary in the specified area and this in turn may have affected blood glucose. Ideally the snacks would have been tested with at least two days between each snack and carried out in duplicate, however due to technician time constraints it was only possible to test each snack once for each participant. Testing on consecutive days was sometimes unavoidable due to changes of participants' diaries. It would have been interesting to carry forward more than two experimental samples to phase two so that different DFs with different water holding

capacities and pasting properties could be tested (for instance inulin and super gum). However time and monetary constraints prevailed.

Determination of the rheological characteristics of the DFs in their food systems would also have been interesting to conduct. In particular the determination of water availability and the effects this had on starch gelatinisation and the enzyme kinetics controlling starch hydrolysis. However limited equipment was available at MMU Hollings and no DSC and no controlled stress/controlled temperature rheometer was available at Hollings. Future work could build on the preliminary results and utilize such equipment in combination with microstructural evaluation of starch structural changes during processing and digestion.

9.3 Summary of results

The preceding pages document the success in attaining these aims. In summary:-

Phase one of this thesis evaluated the potential effects of 8 different commercial and non-commercial fibre rich fractions on the physicochemical and nutritional properties of extruded snack products. The results presented in *Chapter 6* showed that:-

- 1 extruded snack products with added DF could be manufactured to have similar physical properties as that of a non-fibre rich snack product
- 2 the addition of DF to extruded snack products affects the water mobility within a product and this is related to potential pasting properties and viscosity properties of extruded snacks
- 3 generally DF inclusion reduced the amount of glucose potentially released during a starch digestion process and could be used to mediate the glycaemic response of starchy snack products
- 4 not all DF acts in the same manner and the effects on starch digestibility are not necessarily dose responsive
- 5 it is possible to manufacture DF rich extruded snack products with similar consumer qualities as a non-fibre rich product

Phase two of the thesis further investigated the potential of including fibre rich materials (oat bran and psyllium) into extruded snack products at an inclusion level of 15 %. The results presented in *Chapter 7* showed that:-

- 1 it is possible to manufacture DF rich extruded snack products with similar consumer qualities as a non-fibre rich product.
- 2 the relationship between DF content and water mobility is correlated to potential glycaemic response of food products.
- 3 psyllium fibre was effective in significantly reducing the *in vitro* and *in vivo* glycaemic responses of products.
- 4 oat bran inclusion reduced *in vitro* starch digestibility but not *in vivo* glycaemic response.
- 5 no significant correlation could be established between the AUC values obtained using *in vitro* and *in vivo* measurements.

The initial *in vitro* starch digestibility results mimicked the latter *in vitro* starch digestion results from the samples used in the clinical trial. However the difference in results between the *in vitro* and *in vivo* analysis of glucose release as observed in the clinical trial aspect indicate that *in vitro* analysis may not always be an accurate indicator of what happens to products *in vivo*. Never-the-less, the results can be of considerable interest for the food producers that have recently become interested in low GI foods under the pressure of health authorities increasingly concerned about the nation's health in relation to the dietary habits.

Although the results from this thesis can not claim to have found the ultimate answer on the mechanisms involved in decreased starch digestibility when in combination with certain fibres, 'new insights' were provided to widen the knowledge on this subject.

In particular, these sets of experiments showed that the use of DF offers a possible and suitable way to design high DF, low GI functional cereal snack products and to improve their nutritional quality as far as GI is concerned. Thus the physiological benefits of high DF intake can be complemented by the metabolic merits of a low GI diet.

In addition it was demonstrated that certain DF does not diminish the quality, textural properties and sensory acceptability of the final snack products. The final observation to be made is that not all DF behaves in the same manner. Whilst this last point appears very

obvious to the food researcher, it is an important message to the food practitioner in industry who is investigating ways to enhance DF content of food products, and serves as a warning that the formulation of fibres needs to be carefully planned.

9.4 Future work following on from the results of this study

The effect of fibre on starch digestibility and consequently on the GI of starchy foods, is definitely an area that warrants further investigation, especially now with the ongoing consumer awareness on low GI foods coupled with a shortage of DF rich extruded snack products on the market shelf.

This study showed that a reduction in the GI of starch foods could be without loss of consumer acceptance. It would be therefore interesting to determine the exact mechanisms generating these effects, and also the minimum concentration needed to achieve significant difference. For example, the last chapter focussed on correlations between water mobility and starch degradation so are these effects due to the restricted starch swelling, changes of the product microstructure or include also inhibition of α -amylase? By mapping water mobility during both *in vitro* and *in vivo* glycaemic response protocols, and combining basic starch enzyme kinetic evaluations, one may be able to answer this question once and for all.

Thus further *in vitro* digestion studies on model starch systems are needed to be carried out to investigate the effect of the type of fibre, ratios between starch and fibre and different moisture contents. It would be also interesting to compare these effects in relation to different products structure and different cooking procedures (e.g. different amounts of mechanical shear and thermal cooking during extrusion, probably investigating relationships between SME and product characteristics).

However, the ultimate aim must not be forgotten, and this is to generate knowledge which can be used by the food producers to design good quality fibre enriched cereal products with low GI and acceptable sensory properties. Thus, understanding the interaction between fibre and food components and their behaviour during various processing stages is essential. Recording phase, microscopy, images of starch and various fibre mixtures could prove to be useful. In addition investigations on model systems involving selective DF in combination with starch and gluten could be considered to focus on the effect of DF on

rheological characteristics, water distribution between individual components, food microstructure, starch retrogradation and storage characteristics.

Hopefully all of this information will be of use for food manufacturers and it will potentially lead to a wider range of fibre enriched products becoming part of our daily diet. What is clear is that a number of interesting questions remain to be both asked and answered in the future.

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Appendix

Table 11.1. Torque and pressure of barrel and die during the production of samples to determine the effect of extrusion

Sample	Percentage Torque of Maximum	P die (bar)	Temperature at die (°C)
Control	46	15	160
Inulin 5 %	44	25	155
Inulin 10 %	44	15	155
Inulin 15 %	48	15	155
Oat Bran 5 %	46	15	160
Oat Bran 10 %	44	15	160
Oat Bran 15 %	45	10	155
Mushroom Waste 5 %	54	25	165
Mushroom Waste 10 %	50	20	160
Mushroom Waste 15 %	42	5	160
Gatifolia 5 %	48	15	160
Gatifolia 10 %	44	5	160
Gatifolia 15 %	42	10	160
Super Gum EM10 5 %	52	20	165
Super Gum EM10 10 %	49	10	160
Super Gum EM10 15 %	52	20	160
Barley Flour 65 %	55	30	165
Beta Glucans 5 %	60	40	170
Beta Glucans 10 %	60	20	170
Beta Glucans 15 %	60	40	180
Psyllium 5 %	70	40	170
Psyllium 10 %	60	50	175
Psyllium 15 %	60	50	175

Table 11.2. Torque and pressure of barrel and die during the production of samples to determine the effect of extrusion

Sample	Percentage Torque of	P die (bar)	Temperature at
Control	54	25	155
Psyllium 15 %	54	35	160
Oat bran 15 %	54	30	155

Table 11.3. Percentage moisture of raw mix and extruded samples average \pm SD

	Raw	Extruded
Control	12.45 \pm 0.03	7.07 \pm 0.02
Barley Flour	11.89 \pm 0.76	6.16 \pm 0.04
5% β -Glucan	11.38 \pm 0.16	5.93 \pm 0.05
10% β -Glucan	10.83 \pm 0.19	5.16 \pm 0.06
15% β -Glucan	10.66 \pm 0.53	5.18 \pm 0.21
5% Oat Bran	11.82 \pm 0.14	6.99 \pm 0.03
10% Oat Bran	11.71 \pm 0.63	6.61 \pm 0.05
15% Oat Bran	11.89 \pm 0.59	6.80 \pm 0.07
5% Psyllium	11.52 \pm 0.00	5.78 \pm 0.18
10% Psyllium	11.30 \pm 0.30	5.27 \pm 0.01
15% Psyllium	12.02 \pm 0.88	5.13 \pm 0.12
5% Mushroom Waste	11.14 \pm 0.09	6.43 \pm 0.01
10% Mushroom Waste	11.11 \pm 0.27	6.52 \pm 0.08
15% Mushroom Waste	9.25 \pm 0.09	7.95 \pm 0.01
5% Inulin	11.77 \pm 0.05	7.40 \pm 0.02
10% Inulin	11.23 \pm 0.14	7.39 \pm 0.03
15% Inulin	10.40 \pm 0.03	5.75 \pm 0.13
5% Gatifolia	11.85 \pm 0.11	6.73 \pm 0.01
10% Gatifolia	11.67 \pm 0.01	6.62 \pm 0.05
15% Gatifolia	11.56 0.10	6.57 \pm 0.02
5% Super Gum	11.73 \pm 0.07	6.68 \pm 0.09
10% Super Gum	10.90 \pm 0.20	6.31 \pm 0.08
15% Super Gum	10.35 \pm 0.12	6.01 \pm 0.08

Table 11.4. Percentage dietary fibre content of ingredients of extruded products on dry matter basis.

Ingredient	Insoluble fibre	Soluble fibre	Total fibre
Wheat flour	3.06 ±0.35	2.55 ±0.84	5.62 ±0.59
Barley flour	10.62 ±0.46	5.48 ±0.15	16.10 ±0.31
Mushroom	14.26 ±0.54	14.51 ±0.25	28.77 ±0.39
Psyllium	47.45 ±5.42	33.50 ±7.42	80.95 ±6.42
Oat Bran	3.63 ±0.39	15.97 ±2.35	19.60 ±0.03

Participant Information Sheet
Manchester Metropolitan University
Food and Nutrition Group

Exploiting waste stream and whole grains to enhance the nutritional properties of ready to eat snack products

You are invited to take part in an evaluation of the acceptability of three ready to eat snack samples.

Please take time to read the following information carefully. Please ask the contact personnel if there is anything that is not clear or if you would like more information. Thank you for reading this.

Purpose of the Project

The aim of the Project is to produce ready to eat snack samples with enhanced nutritional properties. We would like to evaluate the sensory acceptability of the samples. The results of the evaluation will be included in the researcher's project or published in an academic journal.

Participation

It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep.

The food samples to be served contain wheat (gluten), maize, oats and psyllium (edible plant fibre).

If you suffer from any food allergies, please do not participate.

What will I have to do if I take part?

You will be asked to complete the Questionnaire using a touch screen computer. The taste panel session will last for approximately 10 minutes wherein you will be given small portions of three ready to eat snack samples and asked to rate them in terms of their appearance, smell, flavour, texture, aftertaste and overall acceptability. Staff will be available to explain the process during the session.

Confidentiality and data protection

Your comments and ratings about the sample will remain anonymous. The data on the Questionnaire will only be used in connection with this project.

Contact for Further Information

Kritika Mahadevan

Email: k.mahadevan@mmu.ac.uk

Consent form 1

Consent Form

Title of Project: **Exploiting waste stream and whole grains to enhance the nutritional properties of ready to eat snack products.**

		Please initial box
1	I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.	
3	I understand that data generated from my participation in this study will be looked at by the researcher. Anonymised data might be included in the researcher's project or published in an academic journal. I give permission for data generated from my participation in this project to be used in this way.	
4	I can confirm that to the best of my knowledge I am eligible to take part given the inclusion criteria (listed below): <ul style="list-style-type: none">• I am of generally good health• I have no food allergies• I am aged between 18 and 65 years	
5	I agree to take part in the above study.	

Name of Participant

Signature

Date

Researcher

Signature

Date

Margaret Brennan

Email: m.brennan@mmu.ac.uk

Food, Tourism, Management Department, Manchester Metropolitan University, Hollings Faculty,
Old Hall Lane, Manchester, M14 6HR

Information sheet 2

Project Title: Creating healthier snacks using plant fibres

This work forms the basis of a PhD project. The number of people eating ready-to-eat (RTE) snacks in the UK has risen in recent years, due to convenience and changes in lifestyles. Many of the current snacks on the markets have a high energy content which leads to rapid rises in blood sugar levels. Plant fibres can help to slow this response which has been found to have health benefits in the short and longer term.

The purpose of the project is to establish the effects eating two different snacks (containing different plant fibres) has on blood sugar levels. This would also be compared with a control snack and standard glucose test – a total of 4 experiments.

Taking part in the study – what would you need to do?

If you would like to take part in the above study the following would need to be carried out:

1. Consent form – If you would like to take part after reading this information sheet and after completing the screening questionnaire (below) you would need to sign and return the consent form to the researcher (M.A. Brennan).
2. Pre-study screening questionnaire – Before you can take part in the present study we need to screen for medications and health conditions i.e. diabetes that could affect your ability to participate. Participants for this study must be healthy, Caucasian, non-smokers, and aged between 18-40 yrs.
3. What happens next? – After completing the screening questionnaire we will agree suitable dates for you to visit Hollings Research Laboratories (ground floor next to reception). You will be asked to attend four morning sessions in total.
4. What do I need to do in the morning sessions?
 - As preceding meals affect your blood glucose levels, you will need to be fasted from 9pm the evening prior to each session. You may, however, drink water, tea or coffee (with milk if you prefer, but no sugar).
 - On arrival at Hollings Faculty you will be weighed and your height measured to calculate your BMI.
 - Once you arrive at Hollings you will complete a phlebotomy consent form and then the first finger prick blood test will be carried out.

Information sheet 2 continued

You will then be given one of three test snacks, or a glucose drink (to be consumed within ten minutes)

- Three more finger prick blood tests will be carried out: at 20 min. 60 min. and 120 min.

This should take about 2-hours (you may bring a lap top if you wish to work)

Note:

All snacks will contain maize, wheat flour and oat products some will also contain psyllium husk. You will be required to eat a 35g portion. Whilst you are at Hollings you are permitted to drink water, but you must consume the same amount for each test.

You will need to attend 4 different mornings in total, spread out over two weeks, consuming a different snack or the standard drink each time. You may withdraw at any time.

Data will be kept anonymous in relation to the data protection act. All data used may be published, however we will ensure the data collected is kept strictly confidential.

As a thank you for your participation you will be awarded a £10 gift voucher for each morning session attended, which on completion of the four visits will allow you to choose either £40 Amazon voucher or a £40 Next voucher.

Contact details:

If you are happy to take part in this study or have any queries, concerns or complaints now or later please contact either:-

Margaret Brennan BSc, MPhil (0161 247 2796 or m.a.brennan@mmu.ac.uk)

Dr Emma Derbyshire BSc (hons), PhD, Rnutr (0161 247 2483 or e.derbyshire@mmu.ac.uk)

Food, Tourism, Management Department, Manchester Metropolitan University, Hollings Faculty,
Old Hall Lane, Manchester, M14 6HR

How to find us:

To find directions to Hollings please follow the link below

<http://www.mmu.ac.uk/travel/hollings/>

Many thanks.

Consent form 2

Consent Form

Project title: Creating healthier snacks using plant fibres.

Please initial

Have you read the information sheet?

Do you understand what the project is about?

Are you aware that you will be weigh and measured and your BMI determined?

Are you aware you need to not eat anything from 9pm the preceding evening?

Are you aware that you must not eat any breakfast on the mornings of the test?

Are you aware that 4 blood samples will be taken by finger prick each time?

Are you aware that you will need to visit Hollings four times?

Are you aware that you need to consume the sample food within 10 min?

Do you understand that you need to consume the same amount of water

during each test?

Do you understand that you can withdraw from the study out at any time?

Are you willing to take part?

If you do want to take part, please write you name below:-

Name of participant

Date

Signature

Name of person taking consent

Date

Signature

Should you have any queries please contact either:-

Dr Emma Derbyshire or Margaret Brennan (**0161 247 2483/2796**)

Or email **m.brennan@mmu.ac.uk**

Food, Tourism, Management Department, Manchester Metropolitan University, Hollings Faculty,
Old Hall Lane, Manchester, M14 6HR

Consent form 3

<p align="center">Participant consent form for taking blood samples</p> <p align="center">Project title: Creating healthier snacks by adding different plant fibres</p>

Date:

Screening ID:	
D.O.B:	Age (yrs):
Body Weight (Kg):	Height (cm):

Your blood test will be carried out by a trained phlebotomist. However before we take your blood sample we need to check your medical history and obtain your consent. Please inform the phlebotomist of any of the following:

Any device in situ ?	Yes	No
Have you fainted in the past when you have had blood taken ?	Yes	No
Do you suffer from anxiety attacks in relation to needles or blood ?	Yes	No
Any other complications i.e. allergies Please state.....	Yes	No

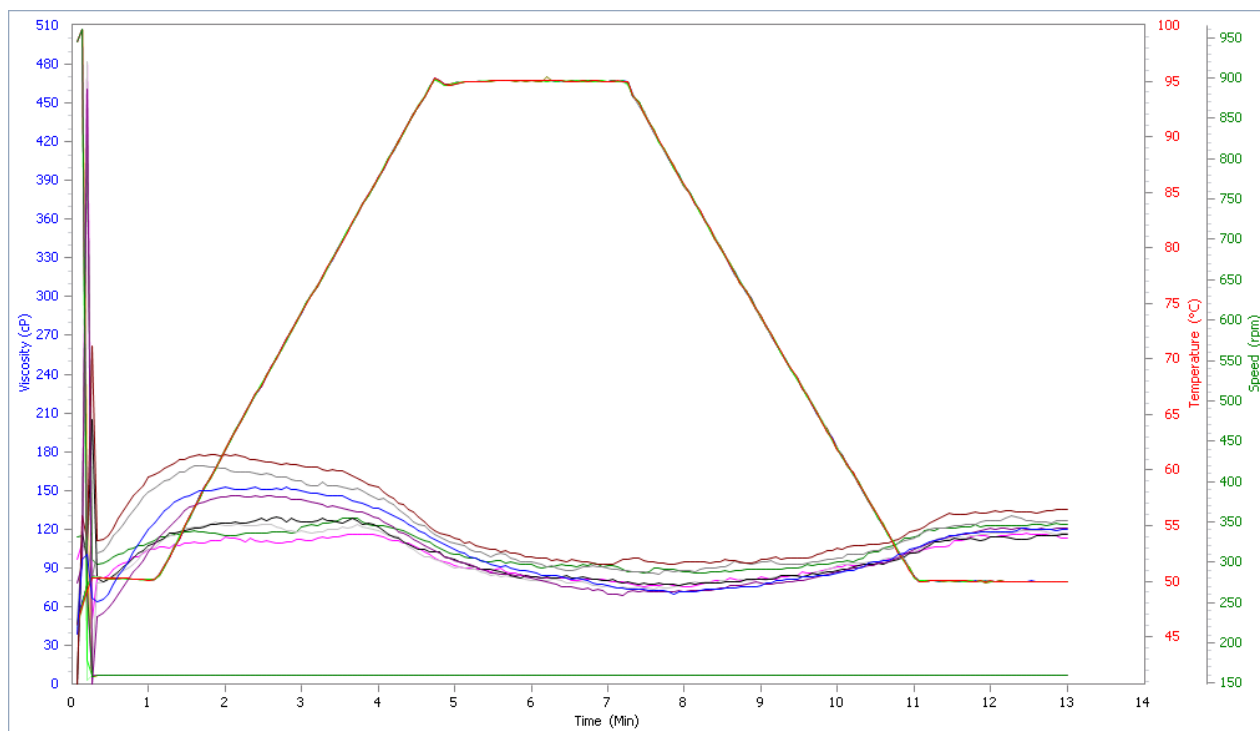
Please talk to the phlebotomist about any concerns you may have. If you prefer to lie down during the procedure please let the phlebotomist know.

Please sign below if you are happy to proceed with the trial.

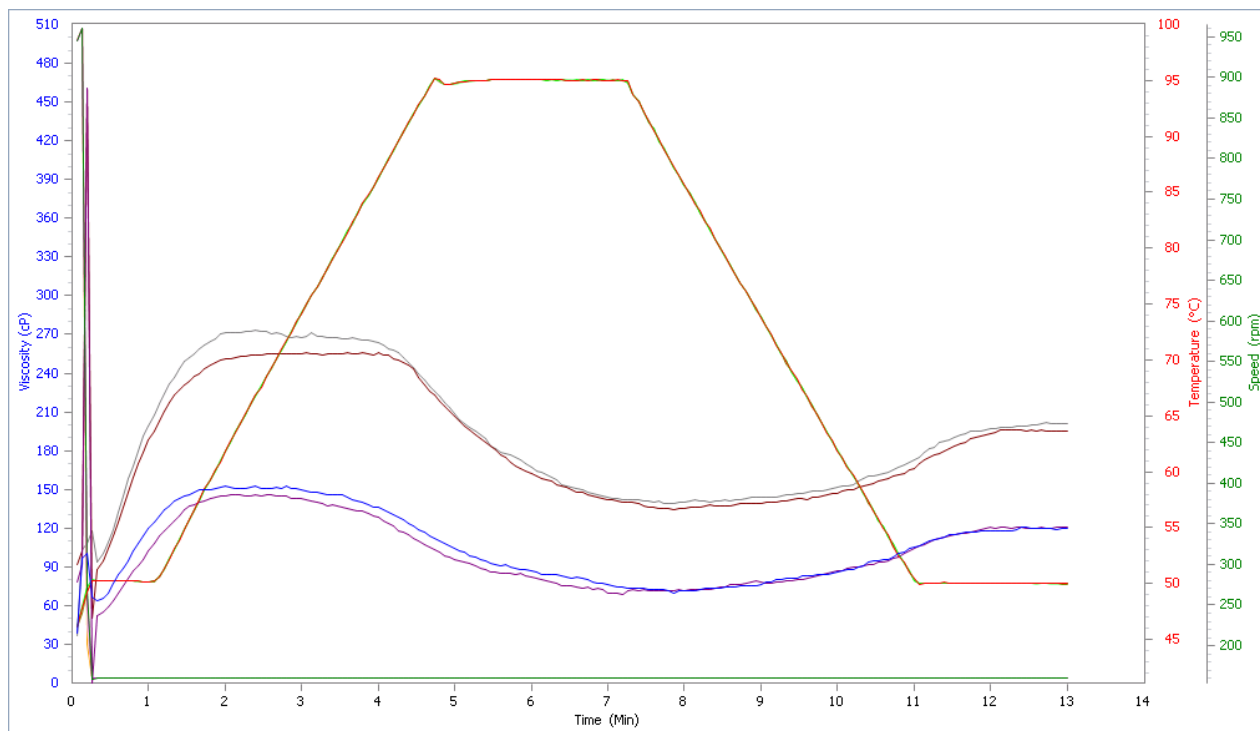
Signature (Participant) Date

Signature (Investigator) Date

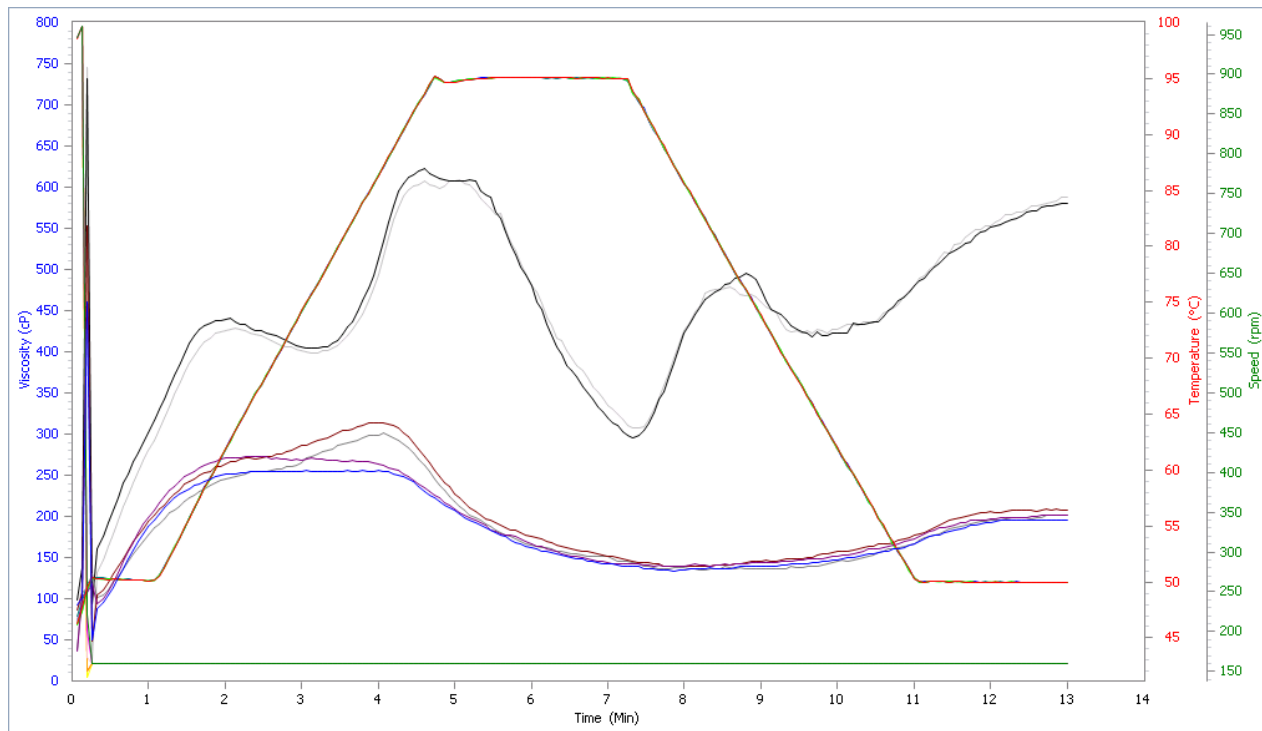
Individual RVA profiles of extruded samples



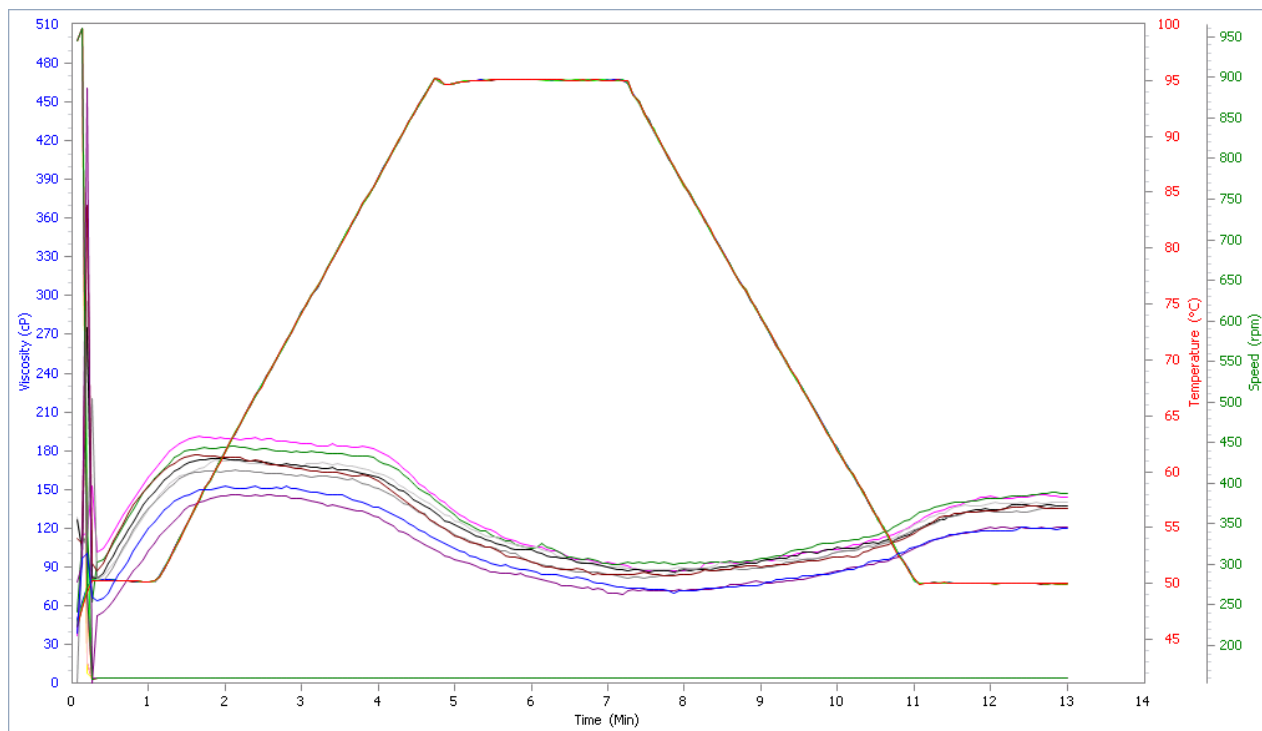
barley flour



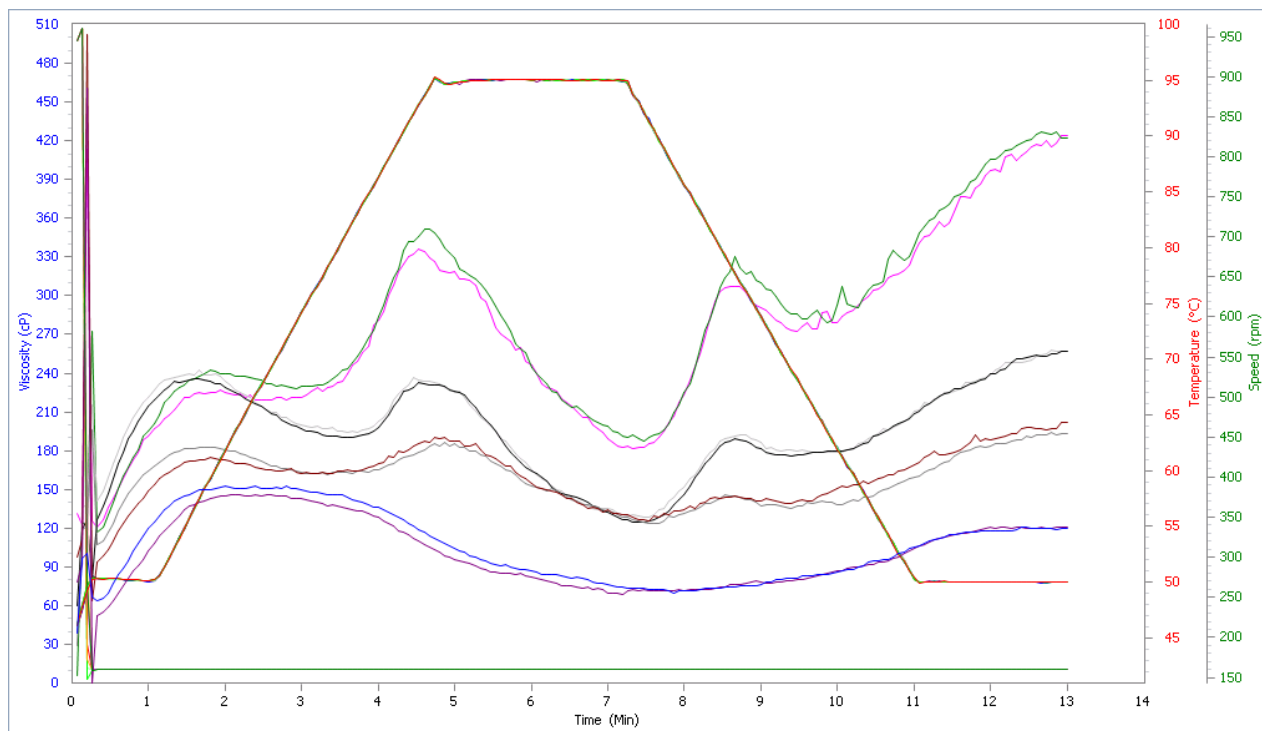
Beta glucan 5 %, 10 %, 15 %



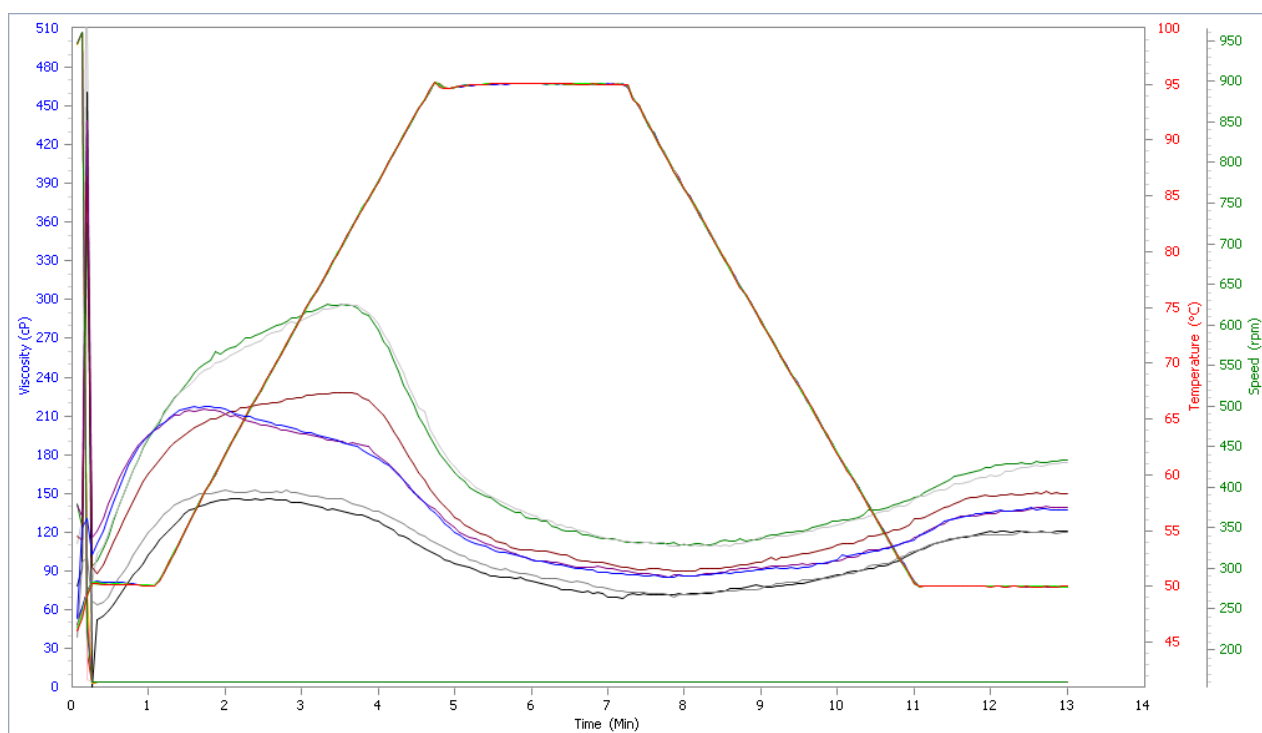
Oat Bran 5 %, 10 %, 15 %



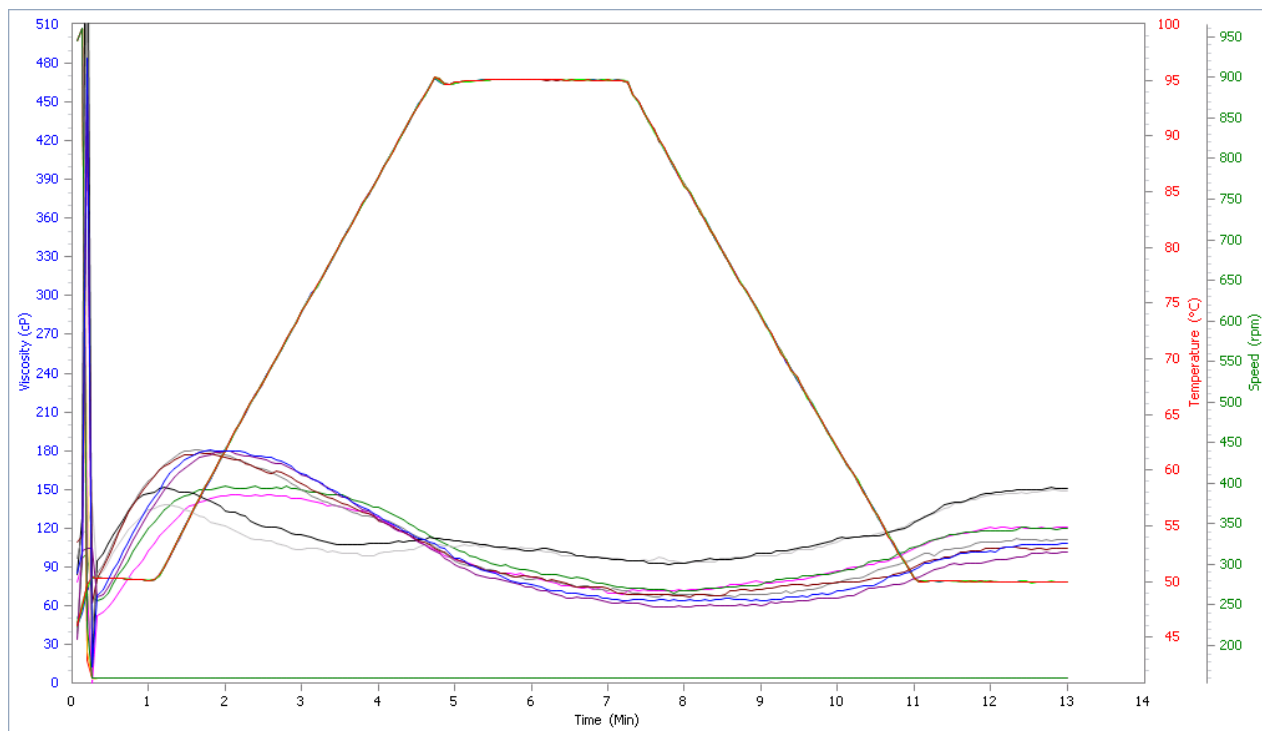
Psyllium 5 %, 10 %, 15 %



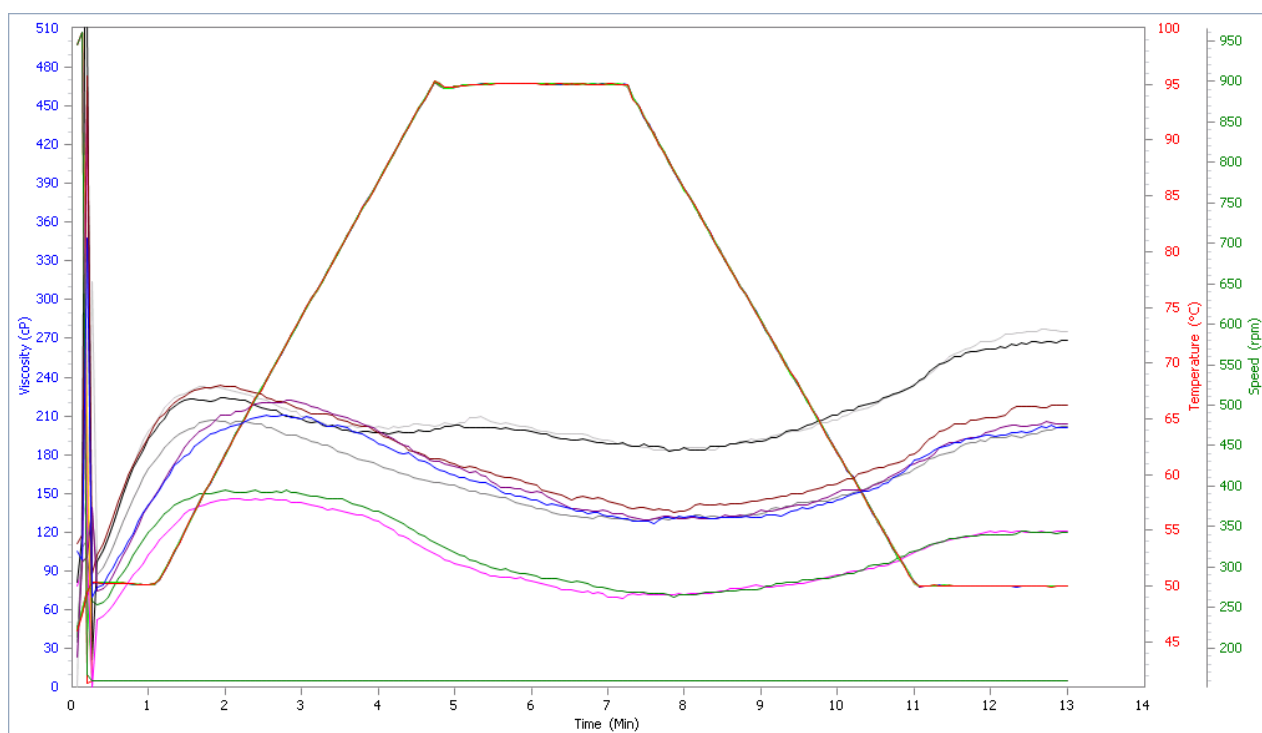
Inulin 5 %, 10 %, 15 %



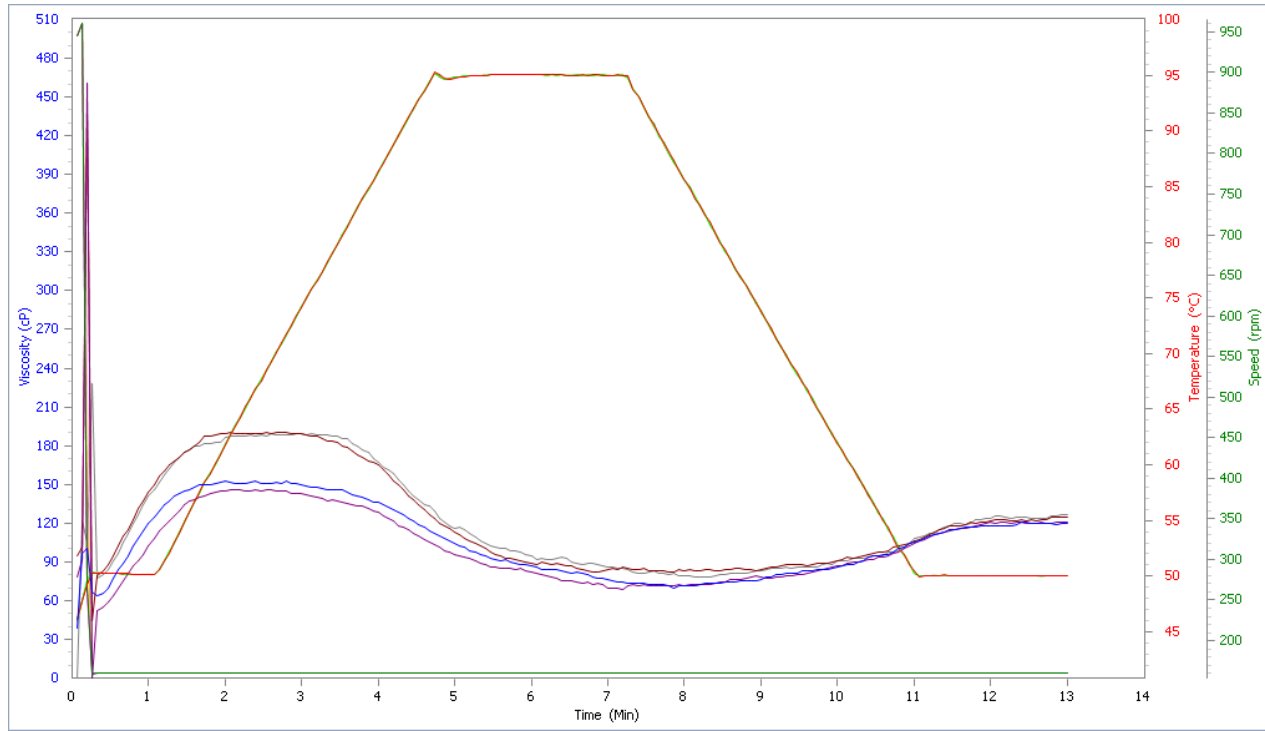
supergum 5 %, 10 %, 15 %



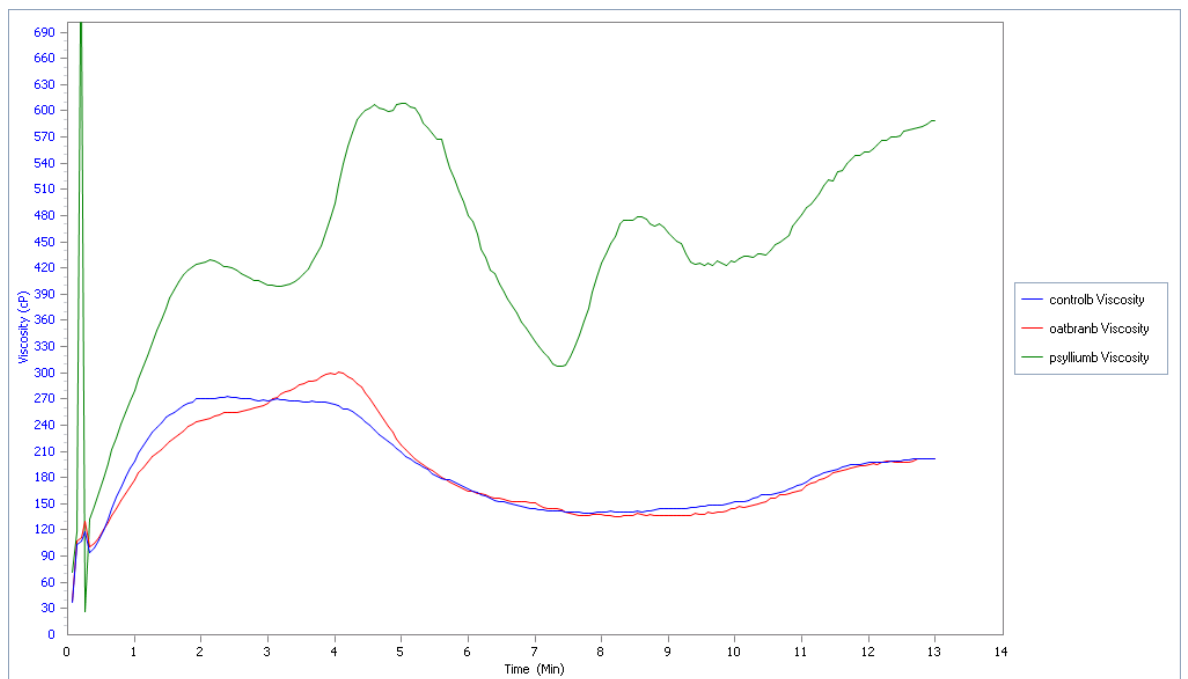
gum gatifola 5 %, 10 %, 15 %



Waste 5 %, 10 %, 15 %



Untoasted and toasted controls



Control Oat bran and Psyllium used in *in vivo* trial

Sensory response sheet 1

+ F.C. 035:1 O.N. 000:1 PAGE 00:1/005 +

YOU ARE PRESENTED WITH SAMPLES OF SNACK PRODUCTS. PLEASE TASTE EACH SAMPLE AND GIVE YOUR OPINION USING THE BOXES BELOW.

THANK YOU

372

	Hard											Soft
HARDNESS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Low											High
CRISPINESS	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Unpleasant											Pleasant
MOUTHFEEL	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Low											High
ACCEPTABILITY	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

PLEASE LIST BELOW ANY FLAVOUR ATTRIBUTES THAT YOU CAN IDENTIFY:-

- 1
- 2
- 3
- 4
- 5

+

+

Collection of sensory responses

Comments summary

Control

PALE LESS CRUNCHY BLAND SLIGHTLY UNPLEASANT
AFTERTASTE
NOT AS PUFFED BUT CRUNCHY
BETTER FLAVOUR BUT SLIGHTLY UNPLEASANT
AFTERTASTE
BLAND

NOT MUCH FLAVOUR
THE PRODUCT WAS A LITTLE BLAND AND DID NOT
HAVE MUCH FLAVOUR
TASTELESS
NOT AS NICE
DID NOT LIKE IT
LIKE EATING PAPER
REVOLTING

BLAND
BLAND
BLAND

BEST BUT WORK REQUIRED ON TEXTURE
NOT AS MUCH FLAVOUR AS THE FIRST SAMPLE
FLAVOR IS NOT VERY EXCITING IN CONTRAST WITH
THE STRONG SMELL

AGAIN NO TASTE OR SMELL
VERY BLAND
VERY BLAND - WORST ONE

NOT MUCH AFTERTASTE
NICE BUT LITTLE BLAND FOR MY TASTE

Psyllium

PREFERE DARKER COLOUR BLAND

GOOD CRUNCHY TEXTURE NEEDS SALT
NICE TEXTURE BLAND FLAVOUR

ACCEPTABLE BUT BLAND

NOT MUCH FLAVOUR
COLOUR TEXTURE AND CRUNCH OK RATHER BLAND TO
TASTE
NO TASTE OR AROMA
OK

NO FLAVOUR LIKE PAPER

6

NO ODOUR BLAND FLAVOUR
BLAND
BITTER AFTERTASTE TOO HARD

PREFERENCE ON SAMPLES 1-3-2

BEST OF THREE BUT WOULD NOT EAT AGAIN

TASTELESS
NO FLAVOUR
VERY BITTER AFTERTASTE
NOT MUCH SMELL NICE CRUNCH
LIKE THESE BETTER

Oat bran

TOO PALE QUITE CRUNCHY BLAND

NEEDS SALT GOOD TEXTURE
BLAND BUT OVERALL OK

ACCEPTABLE BUT BLAND
MORE SALT NEEDED

RATHER BLAND TO TASTE

TASTELESS NO AROMA
FINE

TASTELESS
QUITE TASTY

8

PLEASANT TO LOOK AT BUT TASTELESS
ALL THE SAMPLES TASTED THE SAME ALL HAD NO
SMELL AT ALL

5

TASTE QUITE BLAND
NO TASTE OR SMELL BORING

BLAND
ALL VERY PLAIN
NEEDS COLOUR
NOT MUCH TASTE OR SMELL
OK BUT AGAIN BLAND

Approval form 1



Manchester
Metropolitan
University

ETHICS CHECK FORM

This checklist must be completed for every project. It is used to identify whether there are any ethical issues associated with your project and if a full application for ethics approval is required. If a full application is required, you will need to complete the 'Application for Ethical Approval' form and submit it to the relevant Faculty Academic Ethics Committee, or, if your research falls within the NHS, you will need to obtain the required application form from the National Research Ethics Service available at www.nres.npsa.nhs.uk/ and submit it to a local NHS REC.

Before completing this form, please refer to the University's Academic Ethical Framework (www.rdu.mmu.ac.uk/ethics/mmuframework) and the University's Guidelines on Good Research Practice (www.rdu.mmu.ac.uk/rdegrees/goodpractice.doc).

Project and Applicant Details

Name of applicant (Principal Investigator):	MARGARET BRENNAN
Telephone Number:	ext. 2796
Email address:	m.a.brennan@mmu.ac.uk
Status: (please circle as appropriate)	Undergraduate Student <u>Postgraduate Student</u> (Taught or Research) Staff
Department/School/Other Unit:	Food+Tourism
Programme of study (if applicable):	PhD
Name of supervisor (if applicable):	Emma Derbyshire
Project Title:	Exploiting waste stream and whole grains to enhance the nutritional properties of ready to eat products.
Does the project require NHS Trust approval? If yes, has approval been granted by the Trust? Attach copy of letter of approval.	YES/NO

Ethics Checklist (Please answer each question by ticking the appropriate box)

	Yes	No	N/A
1. Will the study involve recruitment of patients or staff through the NHS, or involve NHS resources? If yes, you may need full ethical approval from the NHS.		<input checked="" type="checkbox"/>	
2. Does the study involve participants who are particularly vulnerable or unable to give informed consent (e.g. children, people with learning disabilities, your own students)?		<input checked="" type="checkbox"/>	
3. Will the study require the co-operation of a gatekeeper for initial access to the groups or individuals to be recruited (e.g. students at school, members of self-help group, nursing home residents)?		<input checked="" type="checkbox"/>	
4. Will the study involve the use of participants' images or sensitive data (e.g. participants' personal details stored electronically, image capture techniques)?		<input checked="" type="checkbox"/>	
5. Will the study involve discussion of sensitive topics (e.g. sexual activity, drug use)?		<input checked="" type="checkbox"/>	
6. Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?		<input checked="" type="checkbox"/>	
7. Will blood or tissue samples be obtained from participants?	<input checked="" type="checkbox"/>		
8. Are drugs, placebos or other substances (e.g. food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?		<input checked="" type="checkbox"/>	
9. Is pain or more than mild discomfort likely to result from the study?		<input checked="" type="checkbox"/>	
10. Will the study involve prolonged or repetitive testing?		<input checked="" type="checkbox"/>	

Ethics Matters

	Yes	No	N/A
11. Will it be necessary for participants to take part in the study without their knowledge and informed consent at the time (e.g. covert observation of people in non-public places)?		✓	
12. Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?		✓	
13. Is there any possible risk to the researcher (e.g. working alone with participants, interviewing in secluded or dangerous)?		✓	
14. Has appropriate assessment of risk been undertaken in relation to this project?	✓		
15. Does any relationship exist between the researcher(s) and the participant(s), other than that required by the activities associated with the project (e.g., fellow students, staff, etc)?		✓	
16. Faculty specific question, e.g., will the study sample group exceed the minimum effective size?	✓		

If you have ticked 'no' or 'n/a' to all questions, attach the completed and signed form to your project approval form, or equivalent. Undergraduate and taught higher degree students should retain a copy of the form and submit it with their research report or dissertation (bound in at the end). MPhil/PhD, and other higher degree by research, students should submit a copy to the Faculty Research Degrees Sub-Committee with their application for registration (RD1) and forward a copy to their Faculty Academic Ethics Committee. Members of staff should send a copy to their Faculty Academic Ethics Committee before commencement of the project.

If you have ticked 'yes' to **any** of the questions, please describe the ethical issues raised on a separate page. You will need to submit your plans for addressing the ethical issues raised by your proposal using the 'Application for Ethical Approval' form which should be submitted to the relevant Faculty Academic Ethics Committee. This can be obtained from the University website (<http://www.rdu.mmu.ac.uk/ethics/index.php>).

If you answered 'yes' to question 1, you may also need to submit an application to the appropriate external health authority ethics committee, via the National Research Ethics Service (NRES), found at <http://www.nres.npsa.nhs.uk/>, and send a copy to the Faculty Academic Ethics Committee for their records.

Please note that it is your responsibility to follow the University's Guidelines on Good Research Practice and any relevant academic or professional guidelines in the conduct of your study. **This includes providing appropriate information sheets and consent forms, and ensuring confidentiality in the storage and use of data.** Any significant change in the question, design or conduct over the course of the research should be notified to the relevant committee (either Faculty Academic Ethics Committee or Local Research Ethics Committee if an NHS-related project) and may require a new application for ethics approval.

Approval for the above named proposal is granted

I confirm that there are no ethical issues requiring further consideration. (Any subsequent changes to the nature of the project will require a review of the ethical consideration(s))
 Signature of Supervisor (for students), or Manager (for staff): [Signature]
 Date: 15/11/2009

Approval for the above named proposal is not granted

I confirm that there are ethical issues requiring further consideration and will refer the project proposal to the Faculty Academic Ethics Committee.
 Signature of Supervisor (for students), or Manager (for staff): _____
 Date: _____

Ethics Matters

Separate page for ethical issues:-

7. All participants will be given id numbers in accordance with 1998 data protection act.
Blood will be disposed of according to 2004 human tissue act.
14. Blood samples will be taken according to 2009 University Health and Safety procedures
Derbyshire E., Evans G., Ashworth J., 2009. Guidelines for blood sampling via venopuncture and cannulation. MMU.
Derbyshire E., Evans G., Ashworth J., 2009. Guidelines for the handling of Human Blood Samples in the laboratory. MMU.
16. Sample size power calculation will be undertaken and sample size will exceed that used in other studies.
Thondre, P.S. and Henry, C.S.K. (2009) High-molecular-weight barley beta-glucan in chapattis (unleavened Indian flat bread) lowers glycemic index. Nutrition Research, Vol. 29, Issue 7, pp 480-486